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The role of intraspecific variation in pest and biological control management

A thesis submitted for the degree of Doctor of Philosophy

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Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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Research is to see what everybody has seen and think what nobody has thought

- **Albert Szent-Gyorgyi**

Abstract

Intraspecific variation is the variation found among individuals of a species and has historically been ignored by ecologists. However, studies are beginning to reveal the importance of intraspecific variation on the ecological performance of individuals and populations; populations have been found to be less vulnerable to environmental change and have more stable sizes when the individuals that composed them are more diverse. Although intraspecific variation is being increasingly explored in ecology, the majority of research has been done using ecological models and observations and have primarily focused on terrestrial vascular plants and freshwater fish. This leaves a gap on experimental research on invertebrate species.

Pest and invasive species, including many invertebrates, are a global problem affecting both the economy and natural ecosystems. Knowledge gaps in the intraspecific variation literature present the opportunity to explore the importance of intraspecific variation in an applied way; to see whether it can be used to help manage and improve pest and biological control species. This thesis therefore aimed to improve our understanding of how intraspecific variation influences population and individual process: (1) within insect species, (2) using controlled experimental studies, and (3) under varying environmental conditions. Each chapter focuses on a different pest or biological control model system to provide guidance for improved pest and invasive species management. Chapter 1 aimed to investigate the effects of intraspecific variation on the pest species, *Sitophilus oryzae,* under novel and heterogeneous environments. Chapter 2 explored whether intraspecific variation could improve the biological control performance of *Aphalara itadori* in the UK, and whether its performance would differ under present and future environmental conditions. Finally, Chapter 3 tested the effects of intraspecific variation on multiple trophic levels using the polymorphic pest species, *Callosobruchus maculatus,* its potential biological control *Dinarmus basalis.* All experimentation was undertaken at the University of Reading Campus and CABI, UK.

Our studies revealed the intraspecific phenotypic and/or genetic variation were not always important for the performance and dynamics of pests and biological controls species under constant or different environmental conditions. Instead, effects varied across experiments and model systems. For example, phenotypic variation did not affect

the overall performance of the pest species *S. oryzae* on the four different grain treatments tested. However, it did affect offspring performance of the polymorphic pest species, *C. maculatus*, with offspring survival varying by morph variant in the presence of its biological control. In terms of genetic variation, the hybrid *S. oryzae* population showed some improved performance (although not for all responses considered), whilst hybrid populations of *A. itadori* were less efficient under both environmental conditions tested. This variation in the importance and consequences of intraspecific differences on the performance of invertebrate species means that we cannot safely conclude that variation is always good, or bad, or irrelevant. Therefore, as a cautionary approach we would recommend ecologists and managers to explicitly consider intraspecific variation within their studies and applied work.

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List of abbreviations

LTLR: long-term laboratory-reared strain

NV: non-variable subpopulation

PV: phenotypically variable subpopulation

STLR: short-term laboratory-reared

SDI: Saturation Deficiency Index

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CHAPTER 1. Introduction

Real individuals are unique combination of traits, some above and some below average. It is time to recognize the uniqueness of the individual and to turn it to our advantage as biologists

- **Albert F. Bennett (1987)**

CHAPTER 1. Introduction

1.1. Variation in the natural world

The fact that individuals from the same species are not identical has long been recognised by scientists (Dall et al., 2012). Intraspecific variation is the term used to describe these differences found within and among individuals of a species, whilst interspecific variation is used to describe the differences that occur between species. Differences within and among individuals of the same species provide the raw material for natural selection to act upon, and therefore intraspecific variation plays a critical part in evolutionary theory (Bolnick et al., 2011). Intraspecific variation can occur at three spatio-temporal scales and ecological levels: (1) variability among populations, (2) variability among individuals, and (3) ontogenetic and seasonal variability within individuals (Albert et al., 2011).

1.1.1. Types of variation and how they occur

Variation can be observed at the level of the genotype and/or phenotype (Raffard et al., 2019). Genetic variation describes the differences amongst individual genotypes and represents the basis for existing biodiversity (Hughes et al., 2008). Phenotypic variation, on the other hand, describes differences in traits: well-defined, measurable properties of organisms that can be detected and quantified (Nock et al., 2016). Traits can be discrete (e.g. the presence or absence of wings) or measurable along a continuous scale (e.g. height and body mass), and includes all the features of an organism other than genotype (West-Eberhard, 1989).

Genetic variation within populations can arise from recombination through sexual reproduction and also evolutionary forces such as migration, genetic drift, and mutations (Albert et al., 2011; Griffiths et al., 2000). In the case of mutations, the level in which genes are expressed and translated can be affected, which can then change structure and function of proteins (Orr, 2005), and therefore some of the observable intraspecific phenotypic variation seen (Angers et al., 2020). Mutations can occur in the soma (non-reproductive cells) or germline (reproductive cells), and any mutations found in the germline are heritable and can undergo natural selection. If the corresponding phenotype of the mutated gene is 'adaptive' (improves an individual's fitness), it is likely to be selected for, whereas if it is 'maladaptive' (reduces the individual's fitness), it is likely to be removed from the population. However, not all mutations lead to phenotypic changes. Silent mutations are changes that do not affect protein structure and function, and therefore, the phenotype (Chamary and Hurst, 2009). This is a form of genetic canalization (suppression of phenotypic variation) as phenotypes are not affected by this type of mutation. In cases like this where canalization occurs, the amount of genetic variation will not be reflected in the amount of phenotypic variation expressed (Hallgrímsson and Hall, 2005). In other words, the amount of genetic variation does not always equal the amount of variation seen.

As mentioned, phenotypic variation is partly shaped by genetic factors, but can also be influenced by environmental factors (Hallgrímsson and Hall, 2005). This 'phenotypic plasticity' in traits can help organisms to survive in fluctuating environments (West-Eberhard, 1989). Phenotypic plasticity seen within an organism can arise due to passive processes (conditions such as temperature, or the amount of salt or nutrients, acting directly on the trait itself and altering enzymes, chemicals and/or cells) or active processes (environmental cues or signals which cause modifications in gene expression

and developmental pathways, and are thought to be adaptive such as wing-length polymorphisms; Angers et al., 2020; Forsman, 2014). The mechanism that allows a single genotype to express different phenotypes in certain environmental conditions, without changing the genetic sequence, is known as epigenetics (Schmid and Guillaume, 2017; Weinhold, 2006), and it can occur through non-coding RNAs, histone modifications and DNA methylation for switching off genes (Duncan et al., 2014).

An individual's phenotype can also change throughout their lifetime (with and without the influence of environmental factors), from the point of fertilisation through to adult. This ontogenetic biological variation or developmental plasticity (Forsman, 2014; Hallgrímsson and Hall, 2005) is regulated by two opposing mechanisms: canalization (which suppresses phenotypic variation, is shaped by evolution and can aid individuals to develop into an optimum adult morphology) and developmental plasticity (which creates phenotypic variation and is useful in populations where offspring are less variable; Hallgrímsson and Hall, 2005). Since the term phenotypic variation relates to the patterns seen in a static observation of a sample, the term phenotypic v*ariability* is therefore used to describe the entire range of possible phenotypes which can occur, but are not necessarily expressed (Wagner et al., 1997; Willmore et al., 2007).

1.1.2. Intraspecific variation in ecological research

Despite the amount of variation that can be seen within a species, intraspecific variation has historically been ignored by empirical and theoretical ecologists (Bolnick et al., 2011), who have instead largely focussed on the effects of interspecific variation ecosystem functioning and dynamics (Figure 1.1). Ecological community and population models created to predict species' abundance dynamics overtime have often ignored

the fact that conspecific individuals are not ecologically equivalent and may not have the same adaptive characteristics (Bolnick et al., 2003; Bolnick et al., 2011). Instead, these models have treated intraspecific variation merely as noise around a species average (Raffard et al., 2017), focusing on trait means and overall population density. This view on the importance of intraspecific variation has, however, changed within the last two decades. Due to biochemical and statistical advances (such as mixed models; see van de Pol and Wright, 2009) ecologists have been offered the ability to measure specialisation at an individual and population level. These advances has also allowed ecologists to explicitly model the effects of intraspecific variation on population, community and ecosystem processes, which have proven some interesting results (see reviews by Bolnick et al., 2011; Dall et al., 2012; Violle et al., 2012; Wright et al., 2016).

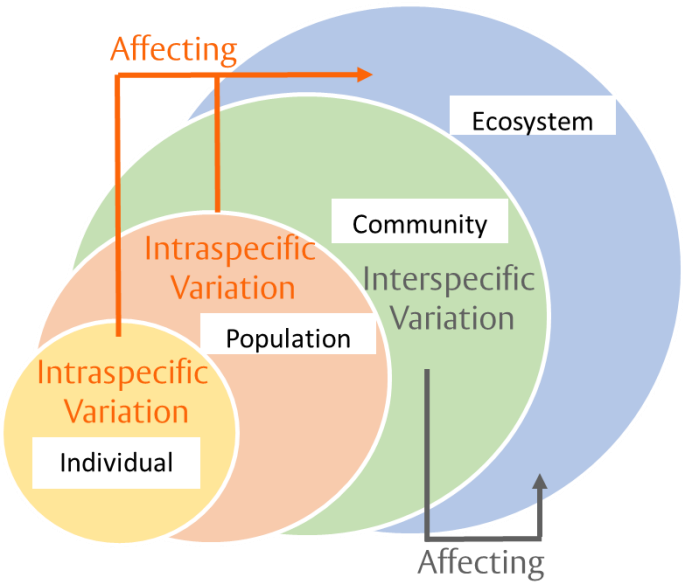

Figure 1.1 Variation at different ecological levels affecting ecosystems. The majority of literature on variation affecting ecosystems look at interspecific variation (grey arrow) whilst there is a need for further studies on intraspecific variation (orange arrows) at the level of individuals and populations.

1.1.2.1. Effects of intraspecific variation in ecological processes

There is now growing interest and evidence showing the importance of intraspecific variation in ecosystems. Intraspecific variation can in fact have large effects on the performance and ecological success of different species. Table 1.1 provides examples of theoretical and empirical studies for different taxa that illustrate the range of ecological effects that have been associated to intraspecific variation at different levels. While not a comprehensive review, these examples highlight the emergence of experimental work that evaluates effects of intraspecific variation in recent years. Examples also show that variation not only affects population dynamics, but also the functioning and structure of communities and ecosystems (Harding et al., 2019). In regards to population dynamics, populations can be less vulnerable to environmental change and have more stable sizes when the individuals that composed them are more diverse (Forsman and Wennersten, 2016). In the case of mammals, intraspecific variation of specific life history traits such as litter sizes and adult body mass, can even act as a buffering system against population and species extinction (González-Suárez and Revilla, 2013).

Table 1.1 Examples of ecological studies in diverse types of organisms that have evaluated the ecological effects of intraspecific variation

Reference	Ecological effects	Model/ observation/ experiment?	Level of intraspecific variation	Type of intraspecific variation	Taxa (genus/ species)
(Post et al., 2008)	Intraspecific variation can affect complex trophic interactions and consequently the regulation of community structure and ecosystem processes	Observation	Population	Genotypic	Fish: Alewives, *Alosa pseudoharengus*
(Albert et al., 2010)	Population differences were a large part of the variability seen. Therefore, giving a species a single trait value and ignoring intraspecific variation can hide the functional responses and effects of a species in different environments	Observation	Population	Phenotype	Plants: 16 different species
(Butterfield and Wood, 2015)	Climate and cultivation affected the functional traits of *Bouteloua gracilis.* This can therefore affect development of commercial seed lines for target restoration sites	Experiment	Population	Genotype & phenotype	Plants: Blue grama, *Bouteloua gracilis*
(Barbour et al., 2016)	Intraspecific genetic variation can be important in structuring ecological networks, and this could further affect the persistence of these networks	Experiment	Population	Genotype	Plants: Coastal willow, *Salix hookeriana* Barratt ex Hooker
(Hart et al., 2016)	Species coexistence can be made more difficult if the intraspecific competitive ability and niche	Model	Individual	Phenotype	Plants: annual species

	variation of a species increases. Intraspecific variation also can increase demographic stochasticity which can contribute to the instability of communities				
(Gibert and DeLong, 2017)	Individual variation in the species' attack efficiency, mutual interference and handling time ability can affect ecological dynamics by making the species more persistent, stable and competitive	Model	Individual	Phenotype	n/a
(Raffard et al., 2017)	Models predict that key ecosystem processes which are correlated to effect traits can be influences by the intraspecific variability in response traits. Furthermore, the composition of response traits could have large impacts on litterstock dynamics and population biomass	Experiment & model	Individual	Phenotype	Invertebrate: Red-swamp crayfish, *Procambarus clarkii*
(Start and Gilbert, 2017)	Variation in the key functional trait activity rate changes prey community structures, trophic cascasdes and ecosystem processes The lowest impacts on prey community structures, trophic cascasdes and ecosystem processes were found for variation within populations were low, and largest among populations, compared to than	Experiment	Within and between populations	Phenotype	Invertebrate: Beaverpond baskettail, *Epitheca canis*

	between co-occurring species or if a predator was present				
(Start, 2018)	Intraspecific differences in activity rate can affect predation of competitors of a different species, and therefore species interactions. However, whether an increase in activity rate was beneficial to the organism, was dependant on whether the organism is a predator or prey in the community (its functional role)	Experiment	Individual	Phenotype	Invertebrates: Beaverpond baskettail, *Epitheca canis* Green darner, *Anax junius*
(Start, 2019)	Mechanisms of intraspecific variation at the individual- and population-level can affect natural selection and ecological interactions within communities	Experiment	Within and between populations	Phenotype	Plant: Tall goldenrod, *Solidago altissima* Invertebrate: *Eurytoma gigantea*
(Start and Gilbert, 2019)	The importance of intraspecific variation compared to interspecific variation on ecosystem functioning depends on the spatial scale considered	Experiment	Within and between populations	Phenotype	Invertebrates: Beaverpond baskettail, *Epitheca canis*

					Dot-tailed whiteface, *Leucorrhinia intacta*
(Zeldin et al., 2020)	Functional trait variation does not always increase when mixing individuals sourced from different populations compared to ones from the same population Variation within populations were found more often to be larger than the variation among populations. This suggests that the diverse genetic material within these populations which have higher within than among population variation could be used	Experiment	Within and between populations	Genotype	Plants: Hairy goldenaster, *Heterotheca villosa* Hoary tansyaster, *Machaeranthera canescens* Tahoka daisy, *Machaeranthera tanacetifolia*

In terms of the functioning and structure of communities and ecosystems, a review on theoretical literature by Bolnick et al. (2011) recognised six mechanisms in which community structures or dynamics can be altered by intraspecific variation: Jensen's inequality, increased degree, portfolio effect, phenotypic subsidy, adaptive eco-evolutionary dynamics and trait sampling (see Bolnick et al., 2011). Intraspecific variation has been found to affect processes such as nutrient and carbon cycles (Lecerf and Chauvet, 2008) and herbivory response (Boege and Dirzo, 2004). The structure of communities can also be affected by intraspecific variation. In experimental studies, significant changes in both the community structure of invertebrate prey and the primary productivity of the ecosystem were detected after manipulating fish phenotypes from several distinct fish populations (Harmon et al., 2009; Matthews et al., 2016). A meta-analysis by Des Roches et al. (2018) also revealed that modifying intraspecific variation can affect communities and ecosystems as much as if species were removed or replaced. Additionally, they found that for trophic cascades and other indirect interactions, intraspecific variation effects can be stronger than interspecific effects. It is, therefore, not surprising that phenotype and genetic intraspecific variation are now being incorporated in the umbrella term 'diversity' (Des Roches et al., 2018).

Since it is now being established that individual traits can affect performance, as well as affect (and be affected by) the composition and interactions within communities and ecosystems (Forsman and Wennersten, 2016), functional ecologists have further classified traits into functional response (how an organism responds to changing ecological factors) and functional effect traits (how the organism's responses affect ecosystem functioning; Díaz et al., 2013; Violle et al., 2007). Although some traits can easily be classified as one or the other, some functional traits can fall into both categories (Raffard et al., 2017). Variation can occur in both functional response and effect traits

and this diversity within and among species in functional traits has become increasingly studied to understand biodiversity (Van Huis et al., 2002).

As a result of the recognised importance of intraspecific variation, the causes, consequences and patterns of intraspecific variation are now a growing field of research in ecology (Bolnick et al., 2011; Dall et al., 2012; Forsman and Wennersten, 2016; Schirmer et al., 2019). However, there are still some major gaps in the literature.

1.1.2.2. Knowledge gaps

Much of the research on intraspecific variation effects on individual and population performance so far has been done using ecological models (Gibert and DeLong, 2017; Hart et al., 2016). However, there is a need for more empirical studies to test these model and theoretical findings, in order to improve our understanding of how intraspecific variation can affect the ecological processes. So far, most empirical studies have been done through observations (e.g. Albert et al., 2010; Brodersen et al., 2012; Post et al., 2008; Zhao et al., 2014), although studies where variables have been controlled or experimentally manipulated have recently become more common (e.g. Barbour et al., 2016; Butterfield and Wood, 2015; Raffard et al., 2017; Start, 2019; Start and Gilbert, 2017). Experimental studies remain relatively rare compared to observation or modelling work (Table 1.1). Additionally, controlled experimental studies offer the opportunity to test the effects of intraspecific variation on species under varying environmental conditions, which have been hypothesized to module the effects of intraspecific variation on ecosystem functioning (Wright et al., 2016). Understanding these potential changes is key in light of ongoing and predicted anthropogenic climate and habitat changes.

Most studies on intraspecific variation have focused on terrestrial vascular plants and freshwater fish, with a gap in our understanding of other taxa and systems (Des Roches et al., 2018). A more comprehensive knowledge is key to reveal circumstances where intraspecific variation can be important for community dynamics, and to identify generalized effects common to a wide range of organisms and environmental conditions, ultimately offering opportunities for improved conservation and management of biodiversity in the future (Forsman and Wennersten, 2016).

1.2. Future Biodiversity: Pests and Invasive Species

Pests can be defined as any organism that causes harm (i.e. damage, disturb or be an inconvenience) to humans and their possessions (e.g. food, livestock, crops and buildings; Hill, 1997). There are therefore numerous types of organisms that can be classified as pests, depending on what is constituted as “harm”. For example, if we consider negative effects on plant health, in the UK alone there would be over 26,700 species of invertebrate and microorganisms classified as a “pest” (The Pest Reference List; Defra, 2015). Focusing on “harm” that reflects damaging or spoiling agricultural food products, insects, weeds and pathogens are the main groups of organisms that can be considered as agricultural pests (Oerke, 2006). Vertebrates pests, such as birds and mammals, can also cause serious damage to properties and human health (Witmer, 2007). Rodents in particular are a major worldwide pest, predating on native animal and plant species, and contaminating and consuming stored food products (Witmer, 2007). Despite there being a wide variety of pest species, there are a number of characteristics that they have in common, in particular, the ability to withstand a variety of physical environments, which enables pest species to spread easily (Tuda, 2011).

Pest species can be further subdivided into native species and species that have been introduced (intentionally or unintentionally) to a non-native region. There are several different terms that have been used to described the latter: “invasive”, “biotic invaders”, “alien”, “exotic”, “nonindigenous”, “introduced” (see Richardson et al. 2000; Colautti & MacIsaac 2004 for reviews). Although these terms for non-native species mostly have negative connotations, non-native species are actually heavily used in our everyday lives and can help provide food and other necessary resources (Pyšek and Richardson, 2010). It is only when non-native species spread, naturally reproduce and become problematic that they are considered “invasive” (Pyšek and Richardson, 2010). Despite the fact that most non-native species perish during transportation or release (Lodge, 1993; Pyšek and Richardson, 2010), a small percentage of introduced species do establish or invade new habitats, and these are some of the most problematic pest species in the world. For example, globally introduced mallard ducks (*Anas platyrhynchos)* have reduced populations of endemic duck species, such as the Hawaiian duck (*Anas wyvilliana),* through hybridisation (Fowler et al., 2009; Rhymer and Simberloff, 1996), and native fish have been eradicated by non-native fish introduced for food (Wittenberg and Cock, 2001). Henceforth we will therefore be using the term invasive species to define non-native species with large self-sustaining and replicating populations that can spread rapidly over large distances (from the point of introduction) to a non-native landscape by human action, and whose spread and establishment threatens native species, habitats, communities or ecosystems (GB Non-Native Species Secretariat, 2008; Hulme, 2009; Kolar and Lodge, 2001; Mack et al., 2000; Pejchar and Mooney, 2009; Pyšek and Richardson, 2010; Richardson et al., 2000; Williams et al., 2010) (Figure 1.2).

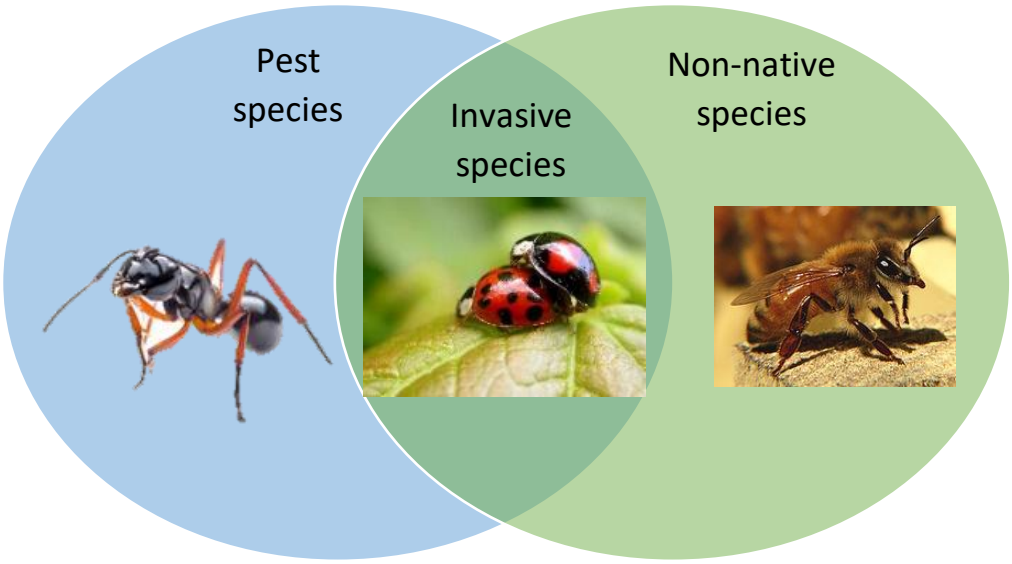

Figure 1.2 Not all pest species are non-native and not all non-native species are pests. UK insect pest species - the black/garden ant, *lasius niger*; UK insect invasive species – the harlequin ladybird, *Harmonia axyridis*; and UK insect non-native species – the Italian bee, *Apis mellifera ligustica* (the widest distributed honeybee in the world)

The most common and most damaging invasive species are mammals, plants and insects (69, 105 and 468 species recorded as invasive respectfully; Global Invasive Species Database, 2020; Wittenberg and Cock, 2001). Species can become invasive either by being preadapted to become invasive (traits that species naturally have that can aid them during the invasive process) or by evolving invasive traits once they have established themselves in the foreign habitat, allowing them to spread more rapidly (Sakai et al., 2001). Although invasive species are not solely associated to human activities, there are strong positive correlations between invasive species and human migration, transportation and trade (Mack, 2000). The end of the Middle Ages and the start of the Industrial Revolution brought about two stages of invasive species introductions, and with increasing modern globalisation (Williams et al., 2010) introductions of non-native species are increasing again, leading to a 'third phase' of invasions (Hulme, 2009). This 'third phase' of invasions brought about by globalisation

is a huge global problem, with invasive species having large economic and ecological impacts as well as affecting human health.

1.2.1. Economic, ecological and health impacts of invasives and pests

1.2.1.1. Economic impacts

Both the global economy and natural ecosystems can be greatly affected by pest species. In terms of economy, animal pests can greatly impact the agricultural industry, creating significant damage to both crops and livestock. Out of all the agricultural pests, weeds cause the most agricultural yield losses (34%), compared to animals and pathogens (18% and 16%, respectively), and globally the overall pre-harvest losses of major crops are more than 25% (Table 1.2; Oerke, 2006).

Table 1.2. Estimated potential loss (%) of six major crops due to pests (*data from Oerke, 2006)*

Major crop	Estimated potential loss (%)
Cotton	80
Maize	31
Potatoes	40
Rice	37
Soybean	26-29
Wheat	50

While impacts are global, China and the United States produce the largest and most diverse commodities worldwide, and are therefore most susceptible to a variety of pests (Paini et al., 2016). Pests that live and develop outside of food manufacturing buildings are usually indigenous, whilst pests that live and develop in food products are often non-native (Kloosterman and Mager, 2013). Since China and the United States trade in large quantities between countries, there is a potential risk that any one of these types of pests will be introduced and could become an invasive pests in other countries further impacting the global economy (Paini et al., 2016).

Food is not the only commodity that humans rely on that can be negatively impacted by the presence of pests. Termites, although necessary for soil ecosystems, can cause permanent infrastructure damage in urban environments by feeding on timber and damaging underground cables and equipment for farming (see Ghaly, 2011). Vertebrate pests have been also found to cause damage to properties as well as causing vehicle or aircraft collisions (Fall and Jackson, 1998). Consequently, millions are spent per annum to prevent these effects. In 2015, the UK alone allocated £19,679,127.77 to public health pest control, with an average cost to a local authority of roughly £47.03 per pest (British Pest Control Association, 2016).

Invasive species alone can lead to major financial and social costs (Pejchar and Mooney, 2009). They can affect costs of ecosystem services related to: provisioning (e.g. food and materials), regulation (e.g. pollination, disease, flood management) and culture (e.g. aesthetics, tourism, leisure activities). For example, the introduction of grey squirrels from northern America cost the UK woodland and forestry industry £10m in damage in 2000, due to diseases brought about from bark stripping. Overall, it is estimated that invasive species decrease 5% of annual production globally (Pimentel, 2002) and in

2007, the Minister for Biodiversity estimated that invasive species control and eradication cost Britain £1.7 billion per annum (Booy et al., 2008; Williams et al., 2010). The global estimate is US$70.0 billion per year and this excludes the US$6.9 billion associated to the impact of invasive species on human health (Bradshaw et al., 2016). There is, however, evidence that some invasive species may be good for the economy (Williams et al., 2010), for example, some invasive plant species could potentially be used as biofuels (Van Meerbeek et al., 2015). Nevertheless, negative effects of invasive species in general far outweigh the benefits.

1.2.1.2. Ecological impacts

Invasive species, in general, negatively impact native ecosystems. With the number of ecosystems dominated by introduced species increasing (Pyšek and Richardson, 2010), this is a major concern. The Millennium Ecosystem Assessment states that invasive species are one of the five major drivers of biodiversity loss and therefore ecosystem services (Pyšek and Richardson, 2010). In terms of the environment, invasive species can damage their non-native habitats through predating, competing, grazing and hybridising on/with native species and also through passing on foreign vectors and diseases (Wittenberg and Cock, 2001). Habitat change due to invasive animal species can lead to the decline and even extinction of vulnerable species. For example, the native British water vole (*Arvicola terrestris*) has rapidly declined due to predation from introduced American Minks (*Mustela vison*), which escaped from fur farms in the 1950s (Carter and Bright, 2003). Equally, diseases spreading from non-native to native species pose a risk, as shown in the decline of red squirrels due to ‘squirrel pox’, carried by the introduced grey squirrels (Chantrey et al., 2014).

Human introduction of invasive species can also lead to homogenisation of biota (Lodge, 1993), destroying ecologically unique biomes. Ecosystem processes can be affected by invasive species through their alterations of the carbon, nutrient cycles and water flow (Clark et al., 2010), which in turn can affect plant productivity, dominant species and the prevalence and survival of native species in an ecosystem (Vitousek, 1990). The changing structure of plants communities by invasive annuals can also impact climate regulation. For example, native perennial sage bushes in the US Great Basin have more net carbon exchange and evapotranspiration than the more fire-prone herbaceous annual grasses, which they are being replaced with as a consequence of invasive annuals (Prater et al., 2006).

1.2.1.3. Health impacts

Pest species can directly and indirectly affect human health. One of the most notorious human diseases spread by pests is the Plague. The devasting loss of lives during the Black Death of 1347–1353 was caused by the bacterium *Yersinia pestis*, which was spread globally by wild rodents and their fleas (Stenseth et al., 2008). A more recent example of pests acting as disease vectors is the brown rat *Rattus norvegicus*, another invasive rodent, which can be commonly found in UK urban development sites and are known to carry Well's and other diseases (Booy et al., 2008). In terms of direct effects, cockroaches are associated with asthma exacerbations and allergies in individuals who have been exposed to pest allergens (Crain et al., 2002), and direct contact with the invasive Giant Hogweed *Heracleum mantegazzianum* can lead to severe burns (Booy et al., 2008). With invasions thought to become more problematic in the future, management strategies are being developed to prevent and control current and future introductions of potential invasive species (Lodge et al., 2006). Worldwide there are now

multi-scale programmes trying to tackle the current and future effects of invasive species (Pyšek and Richardson, 2010).

1.2.2. Current management of pest and invasive species

With pests becoming more of a problem, in 2019 the European pest management services trade association (CEPA) launched its Memorandum of Understanding on Professionalisation of the Pest Management Industry - a document aimed to recognise pest management as a professional and vital industry throughout Europe (CEPA, 2019). The document also aimed for pest management to be included as a professional service sector of the EU regulatory framework. This will mean pest management firms can be audited to a European standard and CEPA certified, in order to make pest management industry professional and sustainable (CEPA, 2019). Additionally, the rise of invasive species in the UK has led to the formation of the GB Non-Native Species Programme Board, which develops strategies to combat non-native invasive species (Williams et al., 2010). Generally, there are four steps to managing invasive and pest species: prevention, early detection/ surveillance, eradication and control.

1.2.2.1. Prevention

Prevention is ‘better than the cure’ (GB Non-Native Species Secretariat, 2008), and it is the least costly and environmentally damaging method in controlling pest and invasive species (GB Non-Native Species Secretariat, 2008; Wittenberg and Cock, 2001). Invasive species in particular can be prevented through regulations with fees and inspections and increased biosecurity standards within trade and transportation. International government legislations, such as the Convention in International Trade in

Endangered Species and Wild Fauna and Flora (CITES), help prevent introduction of invasive species through management of wild animal and plant trade (Booy et al., 2008). More specifically in the UK, the Wildlife and Countryside Act 1981 makes the release of non-native animal species that do not regularly visit the UK naturally illegal. Risk assessments and informing the public through campaigns are also vital in the prevention programmes (GB Non-Native Species Secretariat, 2008).

1.2.2.2. Early detection/surveillance

While prevention is ideal, it is not always successful. Additionally, many species, that have already been introduced and are not problematic now may become problematic and invasive in the future (GB Non-Native Species Secretariat, 2008). Therefore early detection/surveillance of invasive species in the form of surveys on; a) susceptible species, habitats and ecosystems, b) high-risk entry points for invasive species, and c) high conservation value areas, is an important step in determining whether early eradication or containment can be implicated before an alien species establishes itself (Wittenberg and Cock, 2001). The earlier the detection, the greater chance of success, preventing spread and ideally achieving eradication. Food companies regularly use both toxic or non-lethal baits placed around their buildings to detect the presence of rodents and pheromone traps (inspected more than eight times throughout the year) to capture insect pests (particularly insects around stored produce; Kloosterman and Mager, 2013).

1.2.2.3. Eradication

Eradication is the process of removing all individuals or reducing population size to levels at which long-term persistence is unlikely or impacts would be minimal (Myers et al.,

2000), and can be carried out on a small or large scale. Vertebrate and larger pests can be hunted or trapped, and plants and invertebrate species can be mechanically or manually removed, although they can be expensive and labour intensive (Wittenberg and Cock, 2001). For plant species, managing the habitat using sheep and cattle to graze on invasive species or controlled burning regimes can be less labour intensive but may not remove all the below ground material (Wittenberg and Cock, 2001), and in the case of burning regimes, could have negative impacts on the surrounding environment and atmosphere.

Chemical eradication, such as pesticides, is generally used for smaller pest organisms (Bennett et al., 1997) and can be easily applied; however, these methods can be expensive if repeated treatments are needed and can affect non-target species and lead to pesticide-resistance. In terms of pest eradication in food production companies, the regulations of pests and pesticides can differ depending on the country, and even within countries, standard pest control programmes can differ due to variables, such as the building complex, type of food being produced within the factory and customer requirements (Kloosterman and Mager, 2013). There are also environmental and health risks in using chemical pesticides and continuous use of high doses in the long term can eventually lead to pesticide resistance (Lirakis and Magalhães, 2019). Botanical insecticides are a possible alternative to chemical insecticides, as they have little impact on the environment and human health; however, their use in agriculture and industry are still rare (Isman, 2006), since the lack of chemical data and positive controls make them hard to formulate and commercialise (Lengai et al., 2020). Which method to use is determined by evaluating the circumstance, but normally several methods are used in combination, such as with integrated pest management (IPM) programmes where pesticides and biological controls are often used in unison (Pappas et al., 2017).

1.2.2.4. Control

Not all eradications have been successful, and some have been financially and ecologically costly (Myers et al., 2000). For example, Japanese knotweed (*Fallopia japonica*) pesticides and manual removal have had little impact on Japanese knotweed and cause some serious negative effects. Pesticides are generally prohibited near waterways and parks where Japanese knotweed is prevalent (Forman and Kesseli, 2003), particularly within the EU (Pratt et al., 2013). Manual removal, on the other hand, is non-toxic, but generally costly and difficult to do due to the complex underground root system (Forman and Kesseli, 2003). In cases like this, when eradication is not feasible, control of invasive species through biological controls (otherwise known as biocontrols), is an alternative management option (Pratt et al., 2013; Wittenberg and Cock, 2001).

Successful biocontrol programmes use living organisms and do not eradicate the invasive species, but instead aim to reduce its density and/or abundance below an economic or environmental threshold, at which the biocontrol agent can still persist (Eilenberg et al., 2001). Introducing the natural enemies of an invasive species can be sustainable, environmentally cheaper and safer compared to the alternative methods of chemical and manual removal (Pratt et al., 2013). This approach is mostly used for invasive invertebrates and plants (Pratt et al., 2013), with cost benefit analysis showing that it can be a successful management option (Clewley et al., 2012).

There are situations where none of these management options are feasible for economic or practical reasons, in which case, schemes aiming to alleviate the negative effects of the invasive species on important conservation areas become the only option (Wittenberg and Cock, 2001). However, to make any of these management strategies

truly effective in the prevention and management of invasive species, practices need to be shared and implemented internationally (Wittenberg and Cock, 2001).

1.2.3. Improved management? Accounting for intraspecific variation in pest and invasive species

Invasion and pest success are determined at the population, not the species level (Colautti and MacIsaac, 2004) and as discussed above populations of the same species can differ in their dynamics due to the effects of intraspecific variation in traits and reproduction, mortality and migration rates that determine how the population size changes over time and is influenced by the environment. Explicitly considering the effects of intraspecific variation when evaluating impacts of pests and invasives and the potential to use biocontrols is important and can lead to more effective practices.

There is a particular need to gain a better understanding of how intraspecific variation influences dynamics of insect species. Only five out of the 28 of insect orders were represented by work conducted before 2016 (Forsman and Wennersten, 2016) despite the fact that insects make up 80% of the world's species. Many insects play key roles in diverse biological processes and provide important ecosystem services, which we depend on, including: pollination, soil aeration, water purification, biological control, and controlling populations of other trophic species, to name a few (see Losey and Vaughan, 2006). With insect species being vital to the ecosystem and currently threatened by climate change and human activity (Wagner et al., 2021), they are an important group of species to consider.

Previous work has already suggested that considering intraspecific variation could offer useful insights regarding dynamics and management of pest and invasive species. In their review, Forsman and Wennersten (2016) found that higher levels of intraspecific genetic and phenotypic variation are important in the establishment of invasive species, and this can be seen in laboratory, semi-natural and natural conditions, with the largest effects seen in natural experiments. Sakai et al. (2001) also suggested that invasive species ecologists could learn a lot from population biologists to better study and understand the role of intraspecific variation in invasive species research. Knowledge of species' life histories and demographic modelling may help to identify at what point in an invasive species' life history management would have the most impact. Additionally, others have also mentioned how intraspecific variation, in the form of polymorphisms, can be important for pest management (Appleby and Credland, 2001). Given the increasing impact from pest and invasive species, gaining a better understanding of how intraspecific variation influences dynamics and control potential is key. This requires experimental work where variation and environmental conditions are manipulated, and population and individual effects are assessed across different species.

1.3. Thesis model insect systems

Since there is a need for more studies on intraspecific variation in insect species, especially involving pest and biological control species, we decided to investigate intraspecific variation in the insect models: *Sitophilus oryzae, Aphalara itadori, Callosobruchus maculatus* and *Dinarmus basalis.*

1.3.1. Sitophilus oryzae

Sitophilus oryzae (Linnaeus), also known as rice weevils, are a major pest of stored rice and cereals worldwide (Longstaff, 1981). Adults are 2.5-3.5 mm in length and red-brown in colour (Walter Ebeling, 1975). Distinctive features of *S. oryzae* include four faded red or yellow spots on their elytra, and round/irregular punctures on their pronotum (Figure 1.3). Females bore holes within grains into which eggs are deposited. A single female can lay between 300-400 eggs in her lifetime (Walter Ebeling, 1975). In terms of life cycle, *S. oryzae* has 4 instars. At 23-35 °C and 79-87% RH, individuals normally spend 5-6 days as eggs, 16-20 days as larvae and 8-9 days as pupae (Bhuiyah et al., 1990). As adults, *S. oryzae* can live around 114-120 days (Bhuiyah et al., 1990).

Figure 1.3. *Sitophilus oryzae*. Image credit: Domenico Chiarenza

1.3.2. Aphalara itadori

The Japanese psyllid, *Aphalara itadori* Shinji (Hemiptera: Aphalaridae), is a small leafhopper from the superfamily Psylloidea (Figure 1.4). Its eggs are white or orange and turn browner (and almost black in colouration) through each of the five nymphal stages (Figure 1.5). Adults can grow up to 2.5mm in length and are characterised by light and dark brown spotted forewings (Figure 1.4). The developmental time of the psyllid is temperature-dependent, with one generation cycle taking approximately 33 days under 23ºC (Shaw et al., 2009). Adults prefer to lay eggs within the nodes and veins of leaves and each female produces an average of 600 eggs within her two-month lifespan. Nymphs are highly immobile and so will live most of their lives on the host plant they were laid on, with every developmental stage feeding on Japanese knotweed sap for sustenance (Shaw et al., 2009). In Japan, adults use the bark of *Pinus densiflora* and *Cryptomeria japonica* to overwinter (Miyatake, 1973, 2001; Baba and Miyatake, 1982), and therefore it was expected that conifer trees could act as winter refuges in the UK (Shaw et al., 2009).

Figure 1.4. Adult *Aphalara itadori* on invasive host plant *Fallopia japonica.* Image credit: CABI

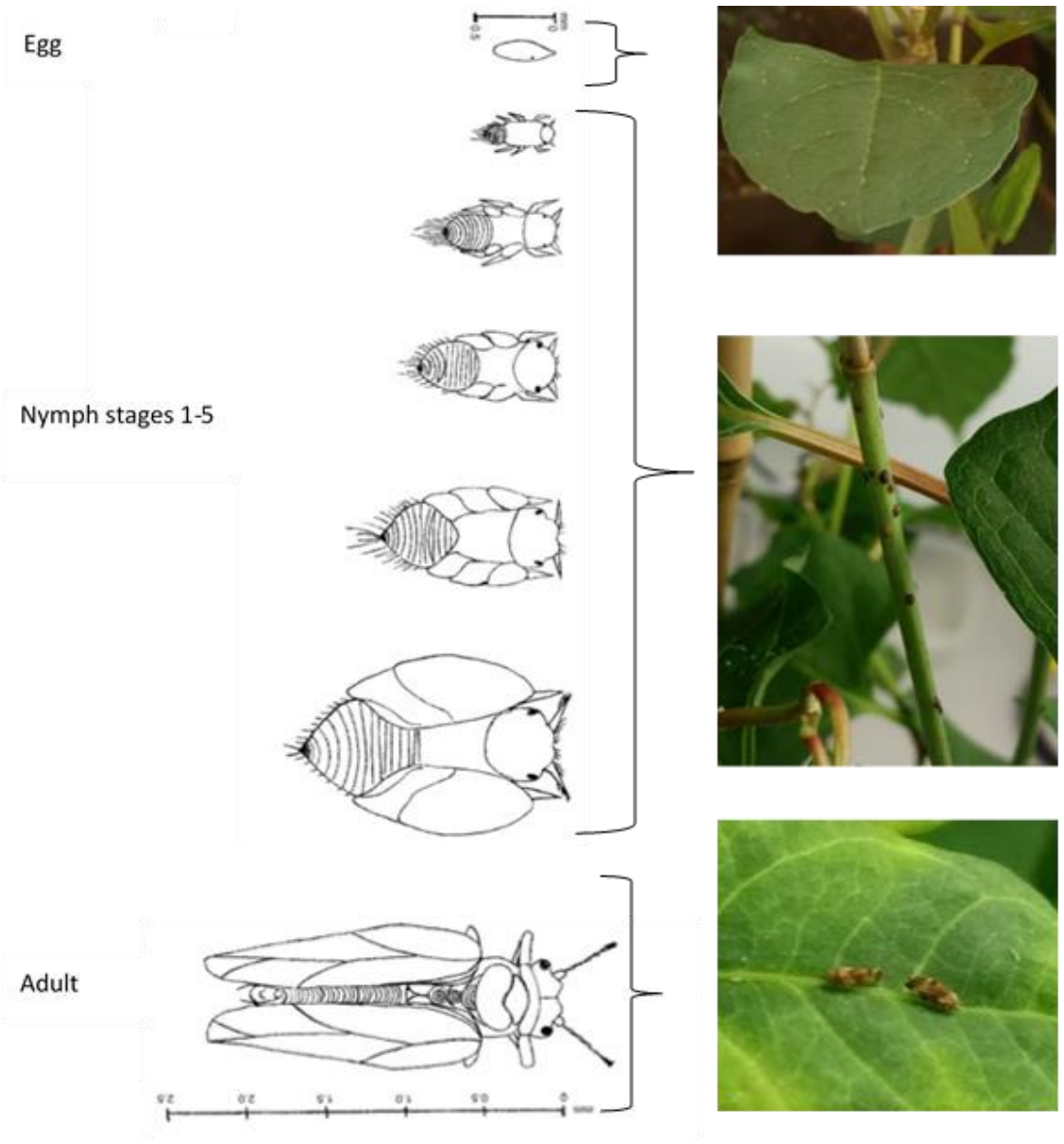

Figure 1.5. Different stages of *Aphalara itadori* development. Eggs are white/ yellow, often found on the nodes and veins of leaves. The five nymphal stage (N1-5) gradually get bigger and browner in colouration as the psyllids develop. Adults are characterised with wings with dark brown patterning. From top down: egg, N1, N2, N3, N4, N5 and adult. (*Adapted from* Shaw, Bryner and Tanner, 2009).

1.3.3. Callosobruchus maculatus *and* Dinarmus basalis

The cowpea weevil *Callosobruchus maculatus* (Fabricius) (Coleoptera:Chrysomelidae) is a global pest of legumes, and can have a huge negative impact on the weight, viability and marketability of their seeds/beans (Bawa et al., 2017; Gbaye et al., 2011). Adults are between 2.0-3.5mm in length, have serrate antennae and can appear as black, brown or brick red in colouration depending on strain, sex and morph (Beck and Blumer, 2014). Females have two distinct dark markings roughly halfway along their elytra whereas males having less distinct markings (Figure 1.6.). In infected bean stores and fields, eggs of *C. maculatus* are firstly oviposited on the outside of beans (Beck and Blumer, 2014). Once the egg develops into a larva, it then bores and feeds on the bean endosperm and embryo. The weevil feeds throughout its four larval instar stages (L1-L4), until it pupates and then emerges as an adult (Devi and Devi, 2014).

Callosobruchus maculatus has been known to display two distinct morphs which differ in morphology, life-history, behaviour, and physiology: a ‘flight’ morph and ‘flightless’ morph (Sano, 1967; Southgate et al., 1957). Flight morphs are more active, have longer wings, are less fecund (laying less eggs per female in a lifetime), have longer life spans and reach sexual maturity later compare to flightless morphs (Utida, 1972). These traits seen by the flight morph correspond to traits used for dispersal. Since flight morphs occur more in high density populations, it is likely these traits help the morph to locate new sites in the field, compared to flightless morph which displays traits that are adapted to life within artificial stores (Bardner, 1982).

Figure 1.6. Adult *Callosobruchus maculatus*. Top row are females and bottom row are males. Left column are examples of the flightless morph and right column are examples of the flight morph.

Dinarmus basalis (Rondani) (Hymenoptera: Pteromalidae) is a solitary ectoparasitic wasp of *C. maculatus* and other similarly related weevil species. The wasp has been known to parasitise *C. maculatus* in both field and storage, laying its eggs in fourth instar larvae (Qumruzzaman and Islam, 2005; Sankara et al., 2014). The wasp then feeds on *C. maculatus* larvae until it emerges as an adult (Qumruzzaman and Islam, 2005; Sankara et al., 2014). Males are distinguished by white markings on their abdomen, whilst females have fully black abdomens (Figure 1.7).

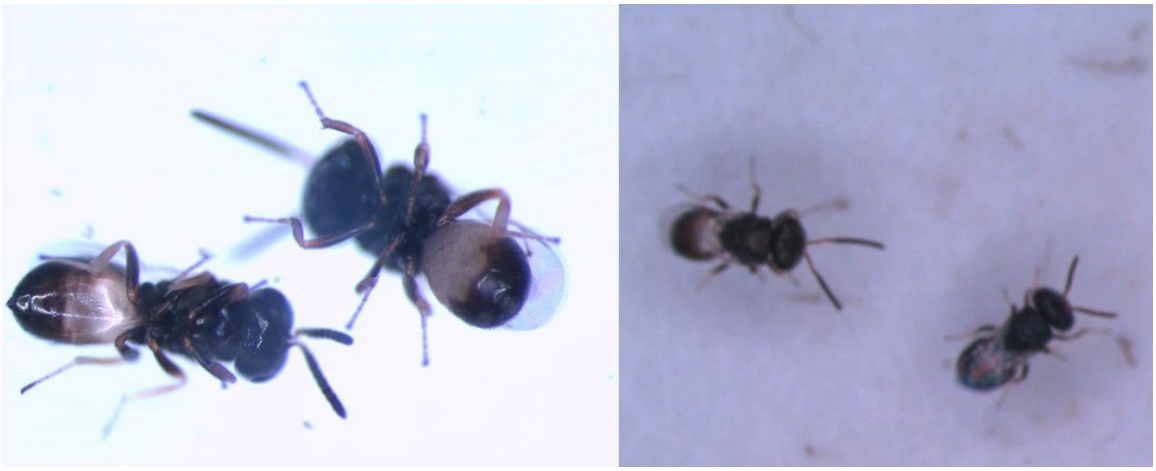

Figure 1.7 Adult male *Dinarmus basalis* with distinctive white markings on the abdomen. Left photograph shows a ventral view and right photo shows a dorsal. Image credit: Kwasi Asante

1.4. Thesis aims and objectives

Reviewing the literature shows a need for improved understanding of how intraspecific variation influences population and individual process: (1) on insect species, (2) in controlled experimental studies, and (3) under varying environmental conditions. This thesis addresses these needs focusing on pest and invasive species to provide guidance for improved management including use of biocontrol agents (Table 1.3). The thesis includes three experimental chapters undertaken at the University of Reading Campus and CABI, UK. Chapters are published or formatted for submission to peer-review journals. The thesis also includes a general discussion (chapter 5). The experimental chapters are:

Chapter 2 – *Intraspecific genetic and phenotypic variation effects on rice weevil individual and population performance.* In dealing with how intraspecific variation can

help with the understanding and management of pest insect species (Aim 1), Chapter 2 explores the role of genotypic and phenotypic variation on the performance of the pest species *Sitophilus oryzae*. The experimental study compares three geographically separated strains and a hybrid strain of *Sitophilus oryzae*, which were further divided into homogeneous and heterogeneous adult body mass test groups. Strains were also tested on four commercially important grains, to explore differences in various environmental conditions (Aim 3). It was hypothesised that higher levels of genetic and phenotypic variation should help improve the performance of *Sitophilus oryzae*, especially on novel and heterogenous grains.

Chapter 3 – *Effect of humidity and temperature on the performance of three strains of* Aphalara itadori*, a biocontrol agent for Japanese Knotweed.* To explore how intraspecific variation effects the establishment and performance of insect biological control species (Aim 2), Chapter 3 (published as Fung et al., 2020) looks at comparing the establishment and performance of two geographically separated strains and a hybrid strain of *Aphalara itadori*, a biological control of the invasive plant *Fallopia japonica.* Strains were also tested under two environmental conditions to assess whether the success of the strains as a biocontrol could be affected by changes in temperature and humidity (Aim 3). It was hypothesised that higher levels of intraspecific variation should help improve the establishment and performance of insect biological control species, especially under more stressful conditions.

Chapter 4 - *The parasitoid wasp* Dinarmus basalis *as an effective biological control for two different cowpea weevil* Callosobruchus maculatus *morphs*. Chapter 4 investigates both Aims 1&2 and tries to establish whether differences in the offspring of the two *Callosobruchus maculatus* morphs would affect the effectiveness of *Dinarmus basalis*

as a biological control. It was hypothesised that the morphology differences life-history between the two *C. maculatus* morphs would be inherited by their offspring and lead to different impacts on *D. basalis* traits and performance, as well as *C. maculatus* consumption of stored cowpea beans.

Table 1.3. Summary of model insect systems used within the thesis and topics investigated

Thesis chapter	Insect species used	Type of insect species	Topics investigated within chapter	Approach used
1	*Sitophilus oryzae*	Pest	Genetic variation	Hybrid vs. single strain populations
			Phenotypic variation	Different morphological and behavioural traits
			Environmental effects	Familiar vs. novel vs. heterogeneous grains
2	*Aphalara itadori*	Biological control	Genetic variation	Hybrid vs. single strain populations and Long lab reared strains vs. short lab reared strains
			Environmental effects	Current vs. future temperature stress conditions
3	*Callosobruchus maculatus*	Pest	Phenotypic variation	Different morphological, behavioural and biochemical traits
	Dinarmus basalis	Biological control		
			Multi-trophic interactions	Performance of pest and biological control, and impact on *Vigna unguiculata*

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CHAPTER 2. Individual traits and population dynamics are affected by genetic but not phenotypic intraspecific variation in rice weevils

Running title: Intraspecific variation effects on rice weevil performance

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CHAPTER 2. Individual traits and population dynamics are affected by genetic but not phenotypic intraspecific variation in rice weevils

Abstract

High intraspecific variation is considered an important factor in enabling populations to persist in novel and heterogeneous environments. We tested how this variation influenced population dynamics of the common pest rice weevil *Sitophilus oryzae,* under laboratory conditions. Three stock populations of *S. oryzae* originating from three continents, as well as hybrid strain created from the three stocks, were used to explore the role of genetic variation. Populations were further divided into homogeneous and heterogeneous adult body mass classes to explore the role of phenotypic variation. Nine replicates of each group were tested on four different commercially important grains. Ten adult mating pairs from each group were firstly placed on each grain type and left to reproduce and deposit eggs for 48 hours, after which adults were removed. Grains were then inspected daily to locate emerging adults, which were removed, sexed and weighed. We used mixed effects regression models to evaluate how variation (genetic and phenotypic) and environment (grain) affected individual (development time, body weight, growth rate) and population (population size, total biomass and body weight variability) characteristics. Phenotypic variation had no consistent effect on *S. oryzae* performance contrary to our expectation that variation would be favourable in novel environments. Genetic variation influenced most characteristics, but we did not find a consistent benefit of increased genotypic variation with higher fitness in the hybrid strain. The consistent decrease in number of offspring in novel and mixed grains suggests a

possible management strategy to reduce abundance of *S. oryzae* by alternating (growing and storing) different grains to create “novel” environments. The results highlight the importance of considering intraspecific genetic variation effects on population dynamics, which has consequences for *S. oryzae* and also other pest management schemes.

Keywords: *Sitophilus oryzae*; Pest species; Grain pest; Novel environment; Pest management; Hybrid

2.1. Introduction

Differences amongst individuals or populations of a species, known as intraspecific variation, have historically been discounted by ecologists with conspecific individuals often treated as ecologically equivalent (Bolnick et al., 2003). Ecological community and population models generally use trait means and overall population density, ignoring intraspecific variation (Bolnick et al., 2011). However, recent biochemical and statistical advances have allowed ecologists to measure and analyse specialisation at an individual level, exploring how variation may influence population, community and ecosystem processes (Dall et al., 2012). Studies have now found that increased intraspecific variation in heritable traits can greatly benefit populations, in terms of: persistence (Kristensen et al., 2008; O’Grady et al., 2006; Vilas et al., 2006), establishment success (Forsman et al., 2012; González-Suárez et al., 2015), reduction in extinction risk (Fox, 2005; González-Suárez and Revilla, 2013), and population growth (Pelletier et al., 2007). Contrary to what has been assumed, intraspecific variation in some characteristics can be as great or greater than variation among species (interspecific variation), and so it is not surprising that intraspecific variation is now being incorporated in the umbrella term ‘diversity’ (Des Roches et al., 2018).

Intraspecific variation can be described focusing on genetic and/or phenotypic differences. Genetic differences reflect the variation among individual genotypes. Different genotypes found at the population level can occur due to evolutionary forces (migration, mutation, genetic drift, and migration; Albert et al., 2011), and at the individual level due to genetic mixing through meiosis. These differences can then be maintained within a population if they are 'adaptive' (improves an individual's fitness) and/or through negative genetic pleiotropy, which is the trade-off in fitness components (Curtsinger et al., 1994), e.g. lower survival associated with faster development rate. Studies have found that intraspecific genetic variation can have direct and indirect effects on population dynamics within a species (which can also affect how they interact with other species within their ecosystem; Barbour et al., 2016). For example, in social insects, genetic variation can aid in protecting against disease (Lloyd-Smith et al., 2005), and in the honeybee *Apis mellifera*, high genetic diversity increases the productivity and longevity of the colonies (Mattila and Seeley, 2007). In terms of phenotypic intraspecific variation, studies have looked at a range of different traits that influence population dynamics, such as morphology, attack rate on prey, fecundity, survival, competitiveness, thermal optima, vulnerability to predators, parasites and disease (see Bolnick et al., 2011), and even colour (which has been found to be a strong proxy for traits such as disease resistance and diet; see Forsman *et al.*, 2020). One phenotypic trait that has been explored extensively is body size (Bolnick et al., 2011), which has been found to be a reliable proxy for a number of other traits or processes including fecundity in insects(Honěk, 1993) and fish such as sockeye salmon *Oncorhynchus nerka* (Garcia De Leaniz *et al.*, 2007), predation risk in the mayfly *Baetis bicaudatus* (McPeek and Peckarsky, 1998), and extinction risk and establishment success in mammals (González-Suárez and Revilla, 2013; González-Suárez, Bacher and Jeschke, 2015).

Theory suggests that intraspecific variation may be influential under certain environmental conditions, and will have different effects on ecosystem functioning

(Wright et al., 2016). This is particularly relevant now as, due to human activity, altered and fragmented new habitats , for example in urban environments, have become more prevalent (Benton et al., 2006; Ramel, 1998). The rapid rate of these changes is greatly affecting populations globally, making them less genetically variable and therefore, theoretically more vulnerable to other changes in their environment (Oliver et al., 2015). In the case of genetic variability, under climate change, more variable populations are predicted to have an increased chance of containing individuals with advantageous genotypes that could allow population persistence in changed environmental conditions (Oliver et al., 2015), whereas locally adapted, less diverse populations may suffer as they have evolved traits to respond to local stress factors (Benito Garzón et al., 2011). In terms of phenotypic variability under novel conditions, a diverse array of phenotypes can result in more diverse responses which can facilitate adaptation and evolutionary rescue of populations under threat of extinction due to the changing environment (Forsman and Wennersten, 2016).

Many papers have studied the impacts of environmental changes (e.g. climate and habitat changes) on the ecological success of populations and species. Most of these are theoretical or observational, although a review by Forsman and Wennersten (2016), looking at the success of among-individual phenotypic and genetic variation in changing environments, does provide examples of experimental work. For example, experimental studies by Via (Via, 1991; Via and Conner, 1995) and Agashe (2009) on the stored product pest *Tribolium castaneum* showed that genetic diversity can increase population persistence and favour stability in novel environments. Whilst more studies exploring the consequences of variation amongst individuals are emerging (Forsman and Wennersten, 2016), we still have limited understanding of how variation among and within populations of the same species influences individual and population level processes. Here, we contribute to this understanding by investigating individual and population performance of phenotypically variable vs similar sub-populations of

geographically (genetically) distinct strains of the rice weevil *Sitophilus oryzae* across familiar and novel environments. By studying the factors that influence performance in a widespread pest species our results could also have additional applied value for pest management.

Studying the effect of variation in the rice weevil *Sitophilus oryzae* can provide ecological as well as applied/management insights as this species is stored product pest that feeds on commercially important grains such as wheat, barley, rice and corn worldwide (Hill, 1990). *S. oryzae* is a good model organism for several reasons: its "natural" environment can be easily recreated in the lab; its rapid life cycle facilitates testing and assessment of population performance; and there is considerable variation among individuals in morphological and life-history traits including body mass, number of offspring and mortality rate (Holloway et al., 1990). Since studies have shown that populations with increased intraspecific variation perform better long-term (e.g. have increased establishment success, population stability and persistence, reduced extinction risk etc) in novel and heterogeneous environments (see Agashe, 2009; Forsman, 2014a; Forsman and Wennersten, 2016), we predicted that: (1) more phenotypically variable sub-populations will perform better; (2) the hybrid strain would be more genetically variable and perform better; and (3) performance of more variable groups will be particularly better on novel and heterogenous environments (grain media).

2.2. Material and Methods

2.2.1. Weevil Stocks and Maintenance

Three strains of weevils reared at the University of Reading were used. Strains originated from natural populations in three distinct regions: Trinidad (collected in 1980),

Queensland (collected in 1986) and Africa (collected from Tanzania in 1980) and exhibit different life history traits (see methods; Holloway, 1984). Additionally, a new hybrid strain (TAQ) was created five months before the start of the experiment by allowing individuals from the three strains described above to interbreed. Second generation (F2) individuals of TAQ were used for the experiment. We assume that sexual reproduction among strains would lead to increased diversity in the hybrid strain. All strains were kept at 30^0 C and 70% relative humidity before and throughout the experiment. Prior to the experiment all individuals were maintained in wheat grain.

2.2.2. Experimental Design and conditions

We set up a cross experimental design with four experimental grain media and four genetically distinct strains (described above) representing intraspecific genetic diversity, each represented by two subpopulations varying in their levels of phenotypic variation. Grain environments all consisted of 3 g of either a single grain (wheat, maize or rice), or a mixed-grain environment created by combining 1 g of each of the three grain types. Wheat was the standard lab grain whereas rice and maize were novel environments for the tested adults. The levels of phenotypic variation included (1) a non-variable (NV) subpopulation in which the starting adult weevils were in the same weight class which corresponded to the average weight for a sample of at least 750 individuals from each strain prior to the experiment, and (2) a phenotypically variable (PV) subpopulation, in which the starting adult weevils represented a uniform weight distribution covering the range of measured weights in that sample of at least 750 individuals. The average body weight of the subpopulations was the same for both levels of phenotypic variation, but the range of weights varied. We established nine NV and nine PV replicates of each weevil strain for each of the grain environments (total 288 replicates initiated with 5760 adult weevils).

At the start of the experiment, virgin adults from each of the four strains were collected from the laboratory stocks immediately after emergence and were sexed and weighed (Table 2.1). Individuals from each sex and strain were grouped into weight classes within a range of 0.1 mg (e.g. 1.1-1.19, 1.2-1.29) and then randomly assigned to the NV and PV subpopulations and the grain environments. To start a replicate, we placed ten adult females and ten adult males from the same strain and subpopulation into an experimental glass tube (dimensions 50x25mm) that contained 3 g of the assigned grain (N = 9 tube replicates for each unique strain, subpopulation, grain combination). Each tube was sealed with a perforated plastic top. Adults were left to mate and lay eggs for 48 hours, after which they were removed from the grain. From day 25 of the experiment, the grain was inspected daily until no more adults emerged from the grain. All emerging adults from each replicate were removed daily, counted, frozen, and subsequently oven-dried. All dried animals were later sexed and weighed. To manage the workload, we had three start dates (01/06/2016; 05/06/2016; 15/06/2016), on each date we set up 3 replicates per treatment.

2.2.3. Data analysis

We measured weevil offspring performance using individual traits measured for each emerging weevil (henceforth referred to as 'individual level' variables) and population variables measured for each replicate (henceforth referred to as 'population level' variables). Individual level variables were calculated separately for males and females and include dry body weight in mg, development period (time from start of the experiment until adult emergence, in days), and growth rate calculated as $Growth\ Rate = \frac{[log10(body\ weight) - log10\ (0.006885)]}{Development\ Period}$ (Sibly and Calow, 1986). Population level variables include: total population size (total number of emerged weevils per replicate), total

biomass (cumulative dry body weight per replicate in mg), and body weight variation (among-individual body weight variation within replicate reported as the coefficient of variation).

We fitted regression models for each individual and population level response. All models included as fixed predictors phenotypic variation, weevil strain (genotypic variation), and grain environment. We also included total population size as a control fixed predictor in nearly all models, except when predicting total population size and total biomass. For the population level variables body weight variation and total biomass, we included mean development period and percentage of females as fixed factors. All models for individual level responses included replicate ID and start date as a random intercept factors, replicate ID was nested within the start date. All population level models included start date as a random intercept factor.

We used linear mixed effect regression models for most of our variables, fitted with function 'lmer' from package *lme4* (Bates et al., 2015) in R version 3.4.3 (R Core Team, 2017). The models for total population size were fitted using generalized linear mixed effects regression models using the function 'glmer', also from the package *lme4*. We fitted models with predictors as additive effects and models that tested paired interactions between phenotypic variation, strain and grain. Models with interactions were only considered to be supported if the interaction coefficients had P-values < 0.05. We evaluated model assumptions (normality and heteroscedasticity) plotting residuals from tested models. Post-hoc tests were performed to evaluate differences within levels of strain, grain and subpopulation using the R functions 'difflsmeans' and 'lsmeansLT' from package *lmerTest* (Kuznetsova et al., 2017) for 'lmer' models, and 'lsmeans' from the package *emmeans* (Searle et al., 1980) for 'glmer' models.

2.3. Results

In total, 5504 weevils emerged from the 288 replicates (mean = 20 individuals emerging per replicate, range = 2-52). For analyses, we excluded data for one individual whose body weight was recorded as <0.1 mg. This was considered an error as this is an order of magnitude smaller than other weights. The fastest developing weevils emerged 26 days after eggs were laid, whilst the slowest developing weevils emerged after 59 days.

2.3.1. Genetic variation

2.3.1.1. Individual level variables

Genotypic variation influenced all response variables (Table 2.1, Figure 2.1), but the effects were dependent on the grain environment and partly on the level of phenotypic variation (Figure 2.2, Figure 2.3, Figure 2.4, Figure 2A.1, Figure 2A.2 & Figure 2A.3). Contrary to the prediction of improved performance in the hybrid strain TAQ, we found that TAQ performance was often intermediate to that of the single-origin strains. For example, TAQ female offspring were significantly larger than those from Trinidad but no different from the other two strains in NV conditions; while, TAQ females were significantly smaller than those from Queensland but similar to the other lines in the PV level (Table 2A.3). Similarly, TAQ females in wheat and mixture had larger body weights than those from Trinidad but were smaller than those from Queensland, while in the novel grains rice and maize, there were no significant differences (Table 2A.3).

Table 2.1 Anova outputs for linear mixed effects regression models predicting *S. oryzae* individual responses (body weight, development period, growth rate) as a function of the main predictors: phenotypic variation (variable and non-variable conditions), strain (the hybrid strain TAQ, Africa, Queensland and Trinidad), and grain type (wheat, rice, maize and mixture of all three grains). Some models also included as predictors: development period, total population size and body weight. Analyses were done separated for females and males to reflect sex differences in response. We report the sum of squares (sum sq), the arithmetic mean (mean sq), degrees of freedom in the numerator (numDF), degrees of freedom in the denominator (denDF), F-value and P-value for variables. Interaction terms are indicated by two variable names separated by a colon. Significant variables and interactions are highlighted in bold.

Variable	Sum sq	Mean sq	NumDF	DenDF	*F*-value	*P*-value
Body weight - Females						
Phenotypic variation	0.007	0.007	1	220.180	0.288	0.592
Strain	**1.400**	**0.467**	**3**	**310.000**	**18.500**	**<0.001**
Grain type	**15.089**	**5.030**	**3**	**271.470**	**199.372**	**<0.001**
Development period	**0.613**	**0.613**	**1**	**2770.130**	**24.288**	**<0.001**
Total population size	**0.500**	**0.500**	**1**	**198.510**	**19.819**	**<0.001**
Phenotypic variation: Strain	**0.234**	**0.078**	**3**	**219.780**	**3.092**	**0.028**
Strain: Grain type	**0.756**	**0.084**	**9**	**266.590**	**3.330**	**0.001**
Body weight - Males						
Phenotypic variation	**0.117**	**0.117**	**1**	**328.26**	**4.279**	**0.039**
Strain	**1.731**	**0.577**	**3**	**331.5**	**21.160**	**<0.001**
Grain type	**15.211**	**5.071**	**3**	**284.45**	**185.938**	**<0.001**
Development period	**0.158**	**0.158**	**1**	**2458.44**	**5.782**	**0.016**

Total population size	0.081	0.081	1	184.4	2.974	0.086
Phenotypic variation: Grain type	**0.264**	**0.088**	**3**	**280.76**	**3.221**	**0.023**
Strain: Grain type	**0.494**	**0.055**	**9**	**280.49**	**2.012**	**0.038**
Development period - Females						
Phenotypic variation	1.570	1.570	1	246.850	0.176	0.676
Strain	**327.140**	**109.050**	**3**	**249.900**	**12.223**	**<0.001**
Grain type	**1121.800**	**373.930**	**3**	**287.870**	**41.915**	**<0.001**
Total population size	4.560	4.560	1	229.550	0.511	0.476
Body weight	**208.390**	**208.390**	**1**	**2805.180**	**23.359**	**<0.001**
Development period - Males						
Phenotypic variation	19.050	19.050	1	266.490	2.119	0.147
Strain	**325.630**	**108.540**	**3**	**269.270**	**12.074**	**<0.001**
Grain type	**1430.840**	**476.950**	**3**	**292.630**	**53.054**	**<0.001**
Total population size	**41.650**	**41.650**	**1**	**235.750**	**4.633**	**0.032**
Body weight	**46.210**	**46.210**	**1**	**2626.600**	**5.140**	**0.023**
Growth rate - Females						
Phenotypic variation	0.000	0.000	1	250.810	0.216	0.642
Strain	**0.002**	**0.001**	**3**	**249.880**	**22.889**	**<0.001**
Grain type	**0.005**	**0.002**	**3**	**280.270**	**65.083**	**<0.001**
Total population size	0.000	0.000	1	230.650	0.352	0.554
Growth rate - Males						
Phenotypic variation	0.000	0.000	1	267.500	3.744	0.054

Strain	**0.002**	**0.001**	**3**	**267.550**	**19.909**	**<0.001**
Grain type	**0.006**	**0.002**	**3**	**283.140**	**70.552**	**<0.001**
Total population size	0.000	0.000	1	234.560	0.966	0.327

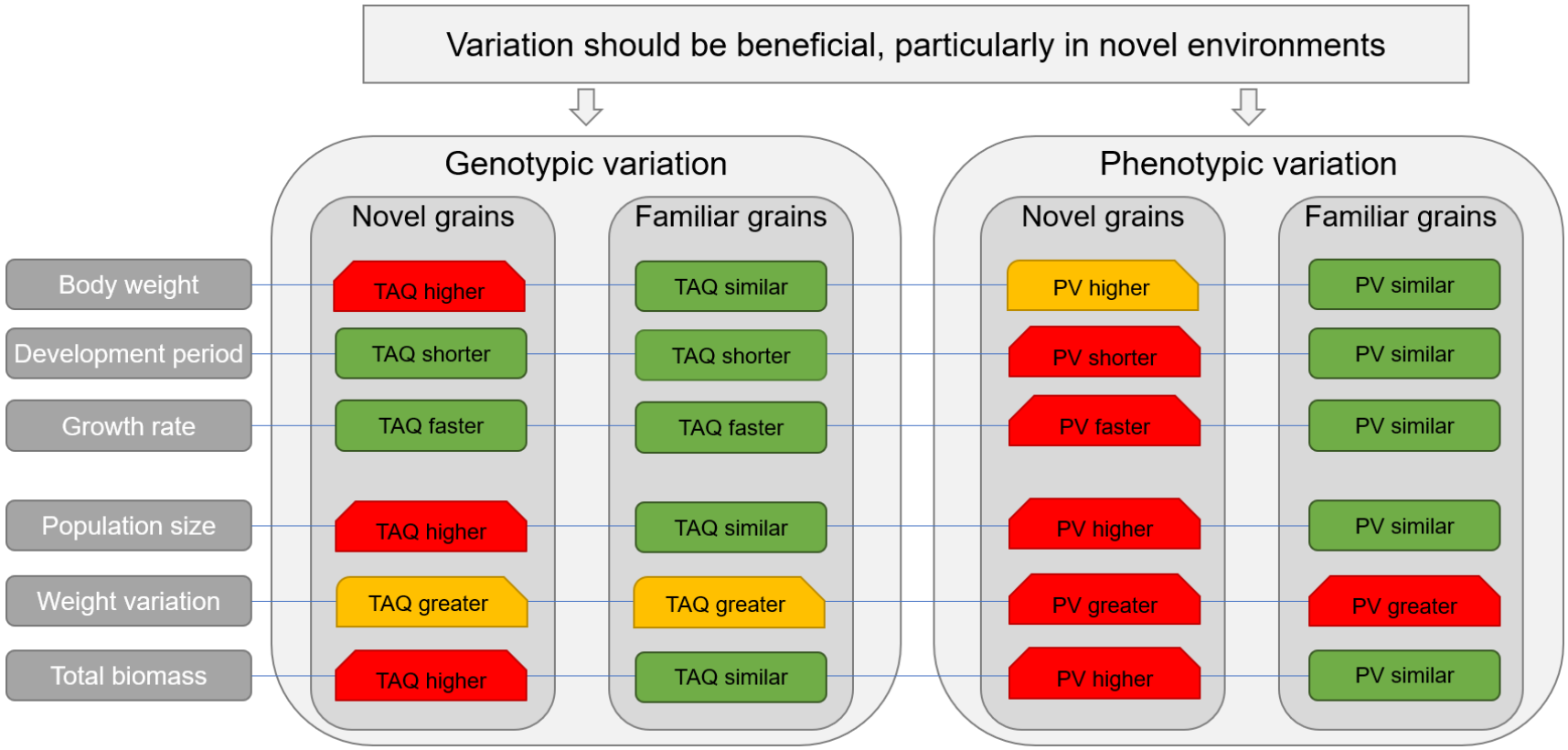

Figure 2.1 Summary of our predictions (written statements) and actual results (box shape and colour) for the variables: (a) body weight; (b) development period; (c) growth rate; (d) total population size; (e) body weight variation and (f) total biomass, for the effects of genetic variation, phenotypic variation and grain type (novel and familiar grains). Statements in the boxes summarize our predictions. Statements in green rectangles indicate our results matched our predictions; in orange rectangles with cut-out top right corners indicate that our results partly matched our predictions; and red rectangles with both top corner cut-out indicate our results did not match our predictions. We use different box shapes as an aid for colour-blind readers.

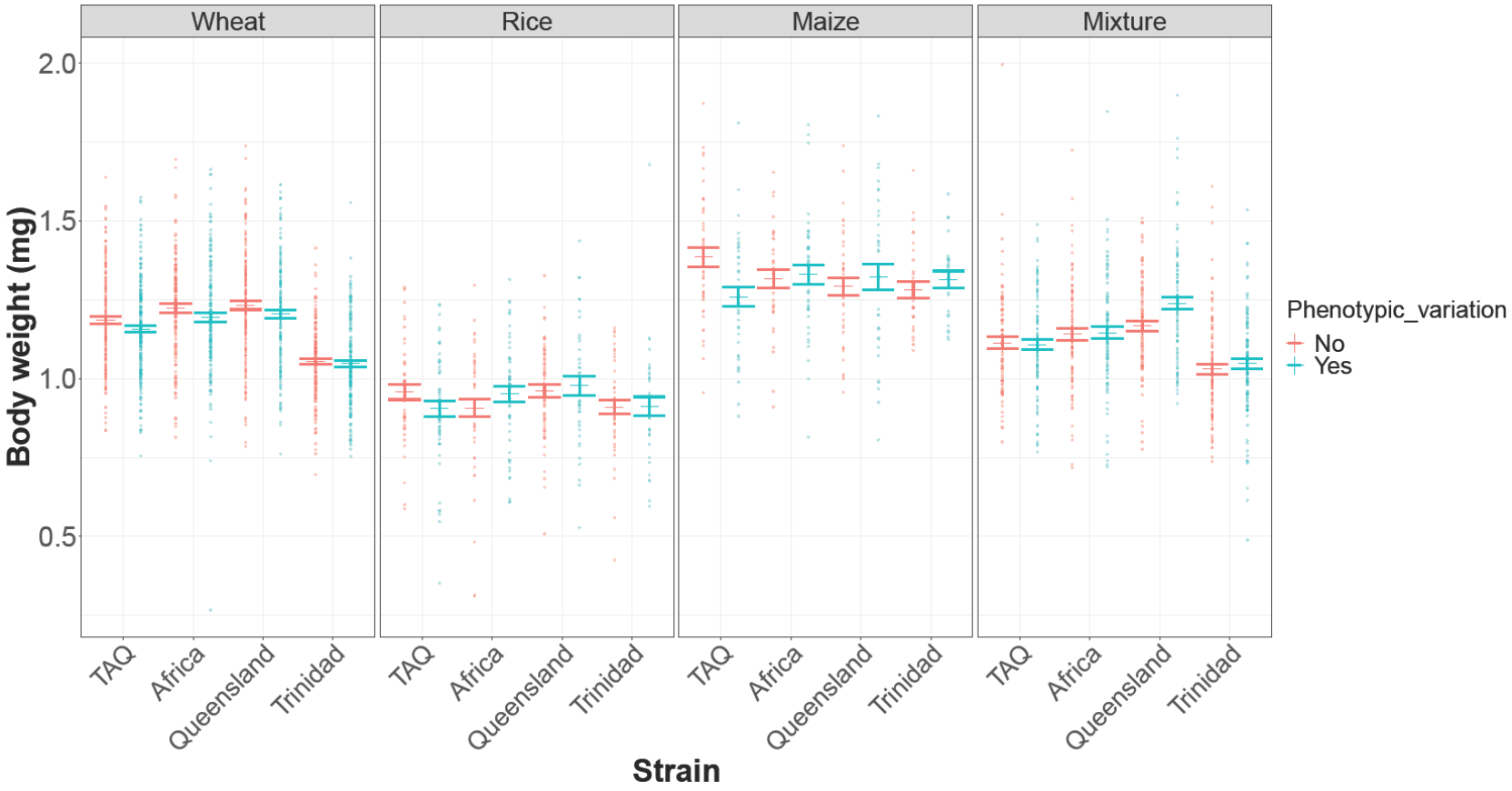

Figure 2.2 The body weight of females for four *S. oryzae* strains (TAQ, Africa, Queensland and Trinidad) on four different grain types; (i) the grain which they were reared in: wheat (ii) novel grains: rice and maize and (iii) a novel and heterogeneous grain: mixed grain. Bars represent the mean ± standard error, whilst data points show the observed values of offspring body weight. Blue bars and points represent predicted body weight phenotypically variable size treatments (V) and red bars and points represent non-phenotypically variable size treatments (NV) of each strain.

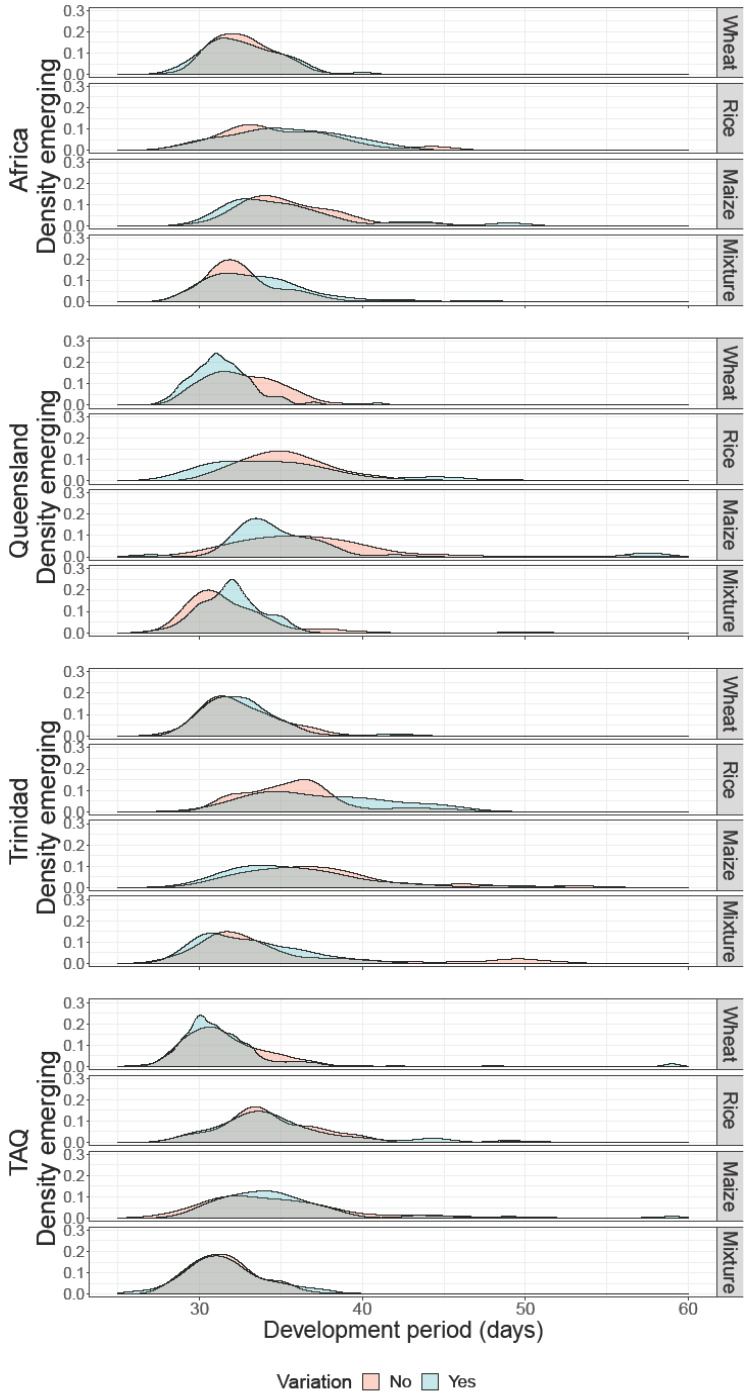

Figure 2.3 The development period of females for *S. oryzae* strains (TAQ, Africa, Queensland and Trinidad) on four different grain types; (i) the grain which they were reared in: wheat (ii) novel grains: rice and maize and (iii) a novel and heterogeneous grain: mixed grain. Curves represent the number of individuals emerging on a given day. Blue curves represent predicted body weight phenotypically variable size treatments (V) and red curves represent non-phenotypically variable size treatments (NV) of each strain.

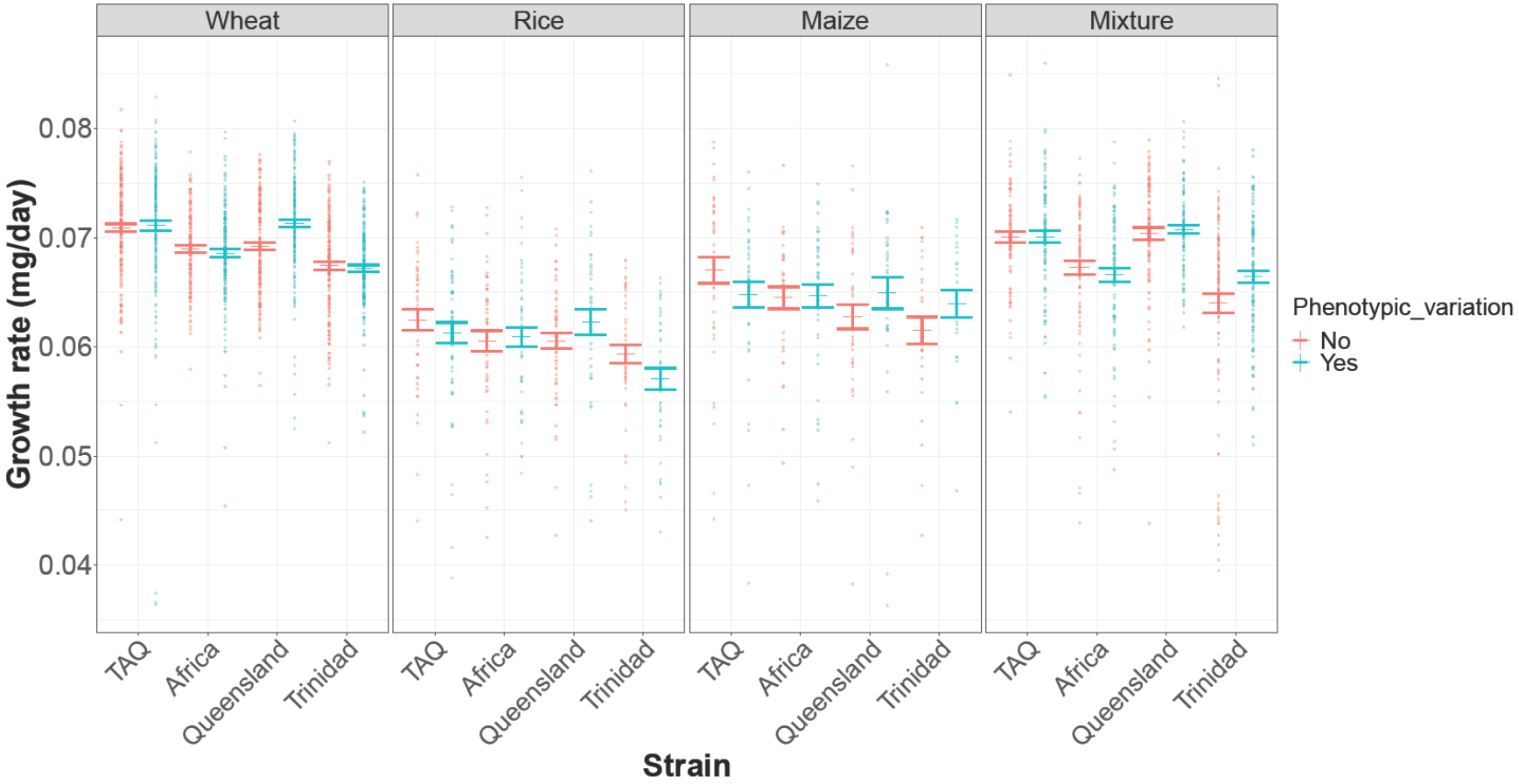

Figure 2.4 The growth rate of females for four *S. oryzae* strains (TAQ, Africa, Queensland and Trinidad) on four different grain types; (i) the grain which they were reared in: wheat (ii) novel grains: rice and maize and (iii) a novel and heterogeneous grain: mixed grain. Bars represent the mean ± standard error, whilst data points show the observed values of offspring body weight. Blue bars and points represent predicted body weight phenotypically variable size treatments (V) and red bars and points represent non-phenotypically variable size treatments (NV) of each strain.

2.3.1.2. Population level variables

Genotypic variation influenced total biomass and population size with effects depending on grain type (Table 2.2 & Table 2.3, Figure 2.1). Partly supporting our predictions, on wheat and maize, TAQ had one of the largest population sizes; however, on mixture grain, it was the Trinidad strain that had the largest population size, with TAQ performing similarly to strains Queensland and Africa (Table 2A.4, Figure 2.5). For total biomass, differences between the strains were only found on wheat where TAQ had an intermediate total biomass (Table 2A.4, Figure 2.6). As predicted, TAQ strain had the most variation in body weights, compared to single-origin strains (Table 2A.3, Figure 2.4).

2.3.2. Phenotypic variation

2.3.2.1. Individual level

Body weight was the only individual level variable affected by phenotypic variation, with differing effects depending on sex, strain, and grain (Table 2.1, Figure 2.2, Figure 2.3, Figure 2.4, Figure 2A.1, Figure 2A.2 & Figure 2A.3). Contrary to our predictions, NV females of the TAQ strain had the largest body weight, irrespective of grain (Table 2A.3, Figure 2.2). Males however, showed the opposite results, with PV subpopulations of all strains having larger mass on heterogenous grains (Table 2A.3, Figure 2A.1). Although this effect of larger male body weight in PV subpopulations agreed with our predictions, no differences were found among PV and NV levels in any of the single-grain environments.

2.3.2.2. Population level

Phenotypic variation had no effect on population size, body weight variation or total biomass (Table 2.2 & 2.3, Figure 2.5, Figure 2.6 & Figure 2.7). Both NV and PV subpopulations performed similarly on all grains (Table 2A.4).

Table 2.2 Anova outputs for linear mixed effects regression models predicting *S. oryzae* population responses (body weight variation and total biomass) as a function of the main predictors: phenotypic variation (variable and non-variable conditions), strain (the hybrid strain TAQ, Africa, Queensland and Trinidad), and grain type (wheat, rice, maize and mixture of all three grains). Some models also included as predictors: development period, total population size and percentage of females. We report the sum of squares (sum sq), the arithmetic mean (mean sq), degrees of freedom in the numerator (numDF), degrees of freedom in the denominator (denDF), F-value and P-value for variables. Interaction terms are indicated by two variable names separated by a colon. Significant variables and interactions are highlighted in bold.

Variable	Sum sq	Mean sq	NumDF	DenDF	*F*-value	*P*-value
Body weight variation						
Phenotypic variation	0.001	0.001	1	273.087	0.770	0.381
Strain	**0.016**	**0.005**	**3**	**272.159**	**3.081**	**0.028**
Grain type	**0.060**	**0.020**	**3**	**235.041**	**11.646**	**<0.001**
Development period	**0.014**	**0.014**	**1**	**236.940**	**8.246**	**0.004**
Total population size	**0.026**	**0.026**	**1**	**78.402**	**15.105**	**<0.001**
Percentage of females	0.002	0.002	1	273.625	1.195	0.275
Total biomass						
Phenotypic variation	22.200	22.200	1	267.010	0.611	0.435
Strain	**310.100**	**103.400**	**3**	**267.350**	**2.848**	**0.038**
Grain type	**21852.500**	**7284.200**	**3**	**267.210**	**200.645**	**<0.001**
Development period	134.900	134.900	1	268.070	3.715	0.055
Percentage of females	**11.200**	**11.200**	**1**	**267.020**	**0.309**	**0.579**
Strain: Grain type	**961.200**	**106.800**	**9**	**267.000**	**2.942**	**0.002**

Table 2.3 Anova outputs for generalized linear mixed effects regression models predicting the population responses of total population size as a function of the main predictors: phenotypic variation (variable and non-variable conditions), strain (the hybrid strain TAQ, Africa, Queensland and Trinidad), and grain type (wheat, rice, maize and mixture of all three grains). We report Chi-square statistic (Chisq), degrees of freedom (DF) and P-value. Interaction terms are indicated by two variable names separated by a colon. Significant variables and interactions are highlighted in bold.

Variable	Chisq	Df	*P*-value
Phenotypic variation	0.830	1	0.362
Strain	**11.856**	**3**	**0.008**
Grain type	**1515.456**	**3**	**<0.001**
Strain: Grain type	**25.113**	**9**	**0.003**

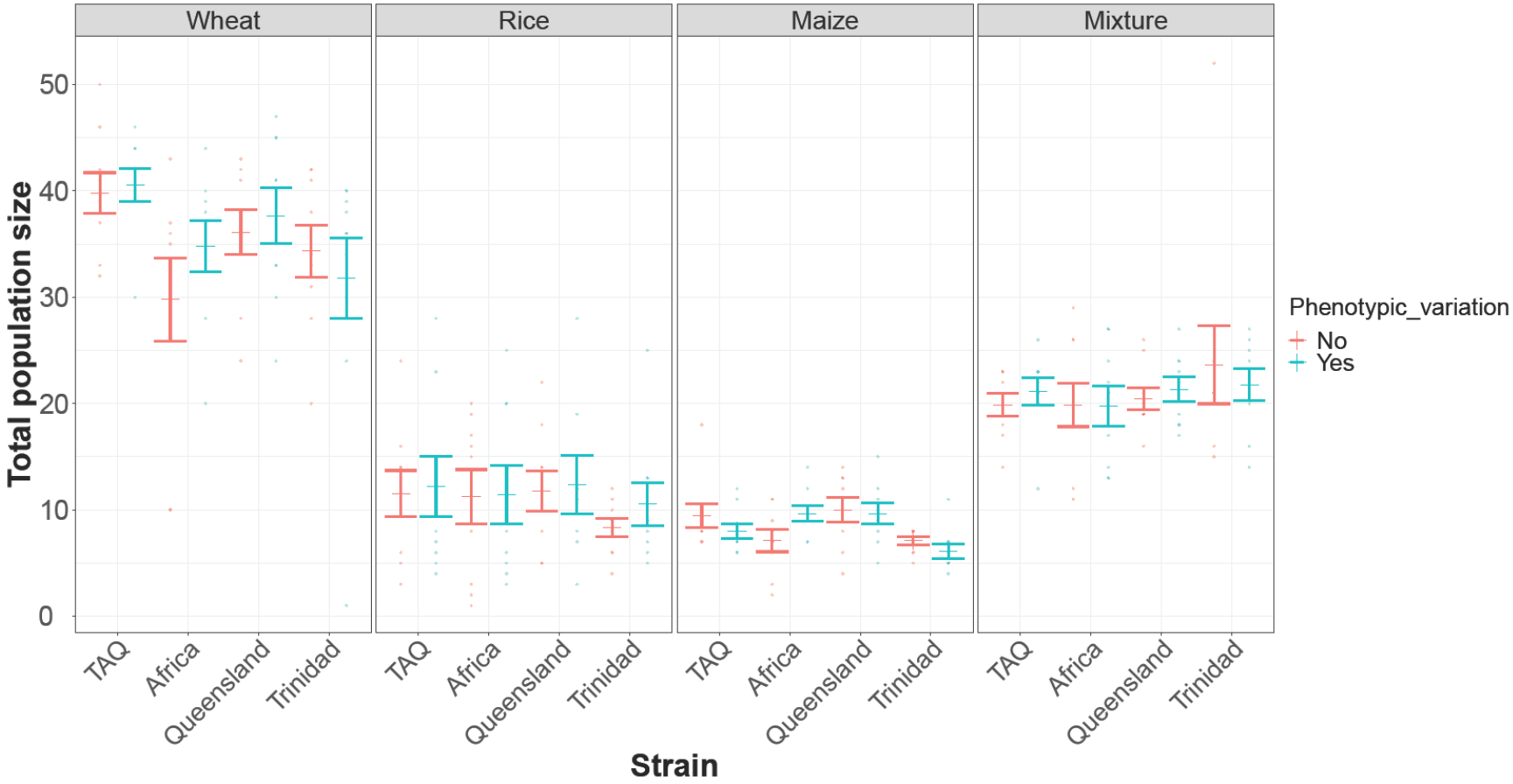

Figure 2.5 The total population size of four *S. oryzae* strains (TAQ, Africa, Queensland and Trinidad) on four different grain types; (i) the grain which they were reared in: wheat (ii) novel grains: rice and maize and (iii) a novel and heterogeneous grain: mixed grain. Bars represent the mean ± standard error, whilst data points show the observed values of offspring body weight. Blue bars and points represent predicted body weight phenotypically variable size treatments (V) and red bars and points represent non-phenotypically variable size treatments (NV) of each strain.

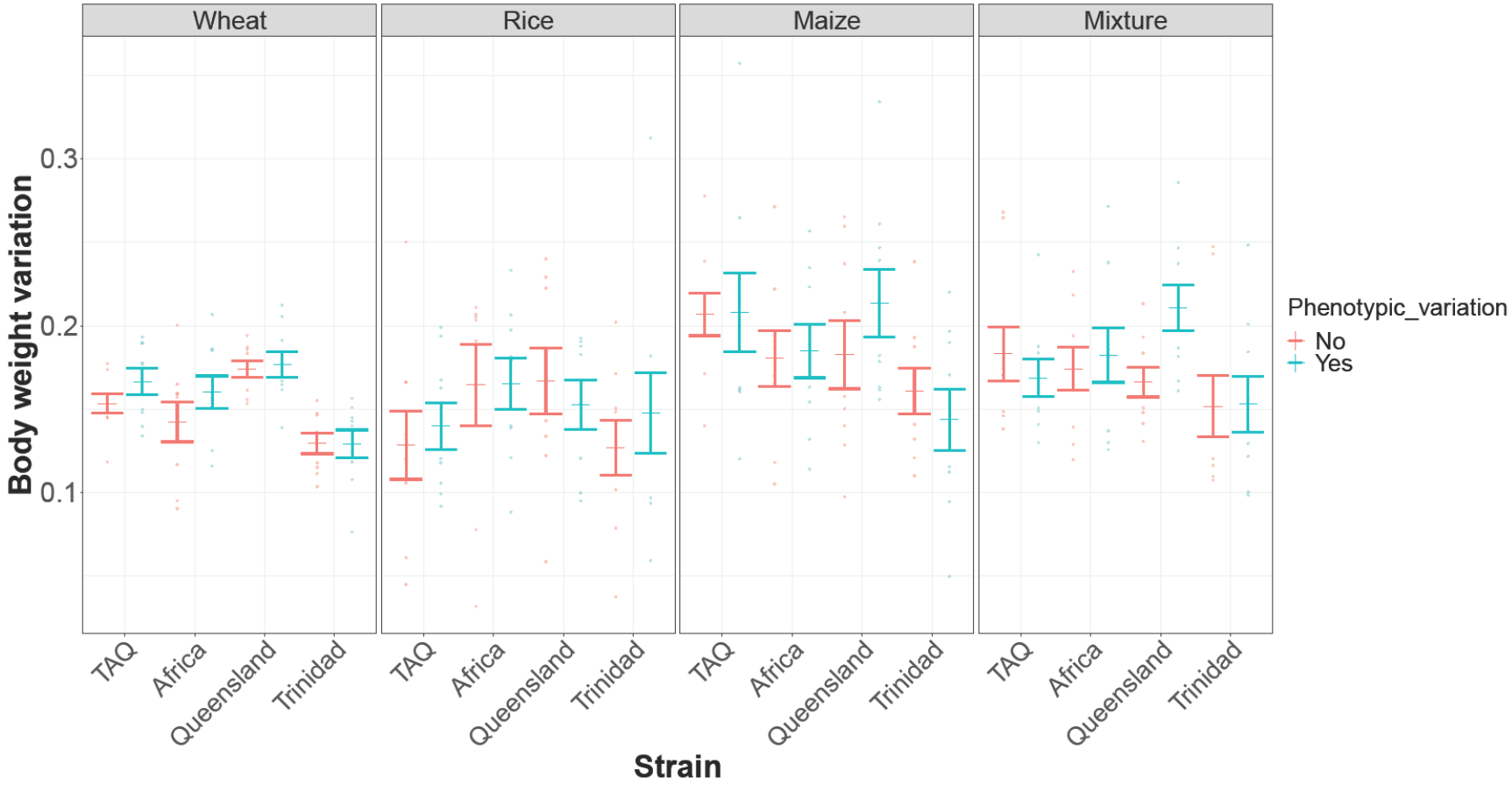

Figure 2.6 Dry body weight variation of four *S. oryzae* strains (TAQ, Africa, Queensland and Trinidad) on four different grain types; (i) the grain which they were reared in: wheat (ii) novel grains: rice and maize and (iii) a novel and heterogeneous grain: mixed grain. Bars represent the mean ± standard error, whilst data points show the observed values of offspring body weight. Blue bars and points represent predicted body weight phenotypically variable size treatments (V) and red bars and points represent non-phenotypically variable size treatments (NV) of each strain.

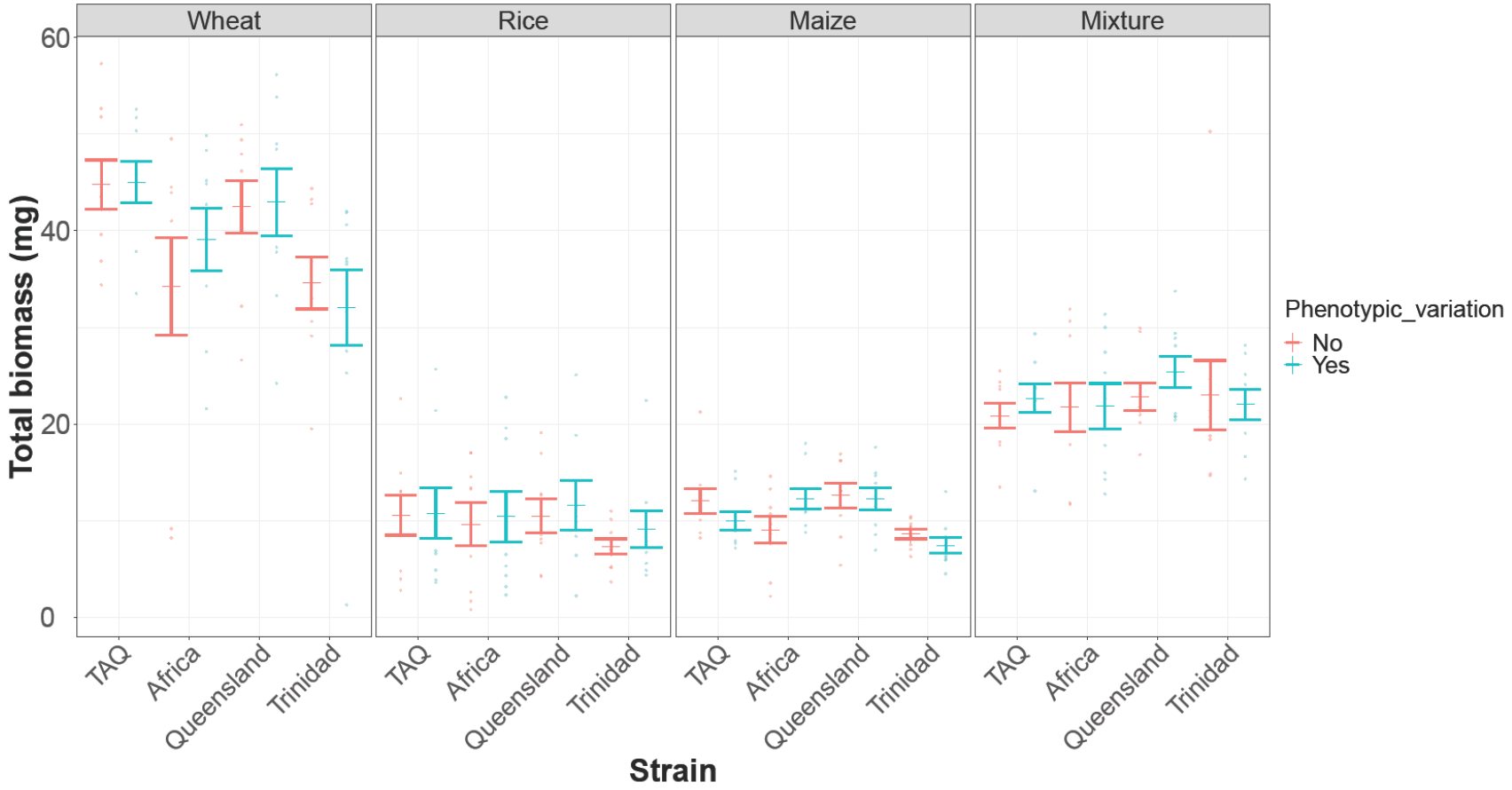

Figure 2.7 The total biomass of four *S. oryzae* strains (TAQ, Africa, Queensland and Trinidad) on four different grain types; (i) the grain which they were reared in: wheat (ii) novel grains: rice and maize and (iii) a novel and heterogeneous grain: mixed grain. Bars represent the mean ± standard error, whilst data points show the observed values of offspring body weight. Blue bars and points represent predicted body weight phenotypically variable size treatments (V) and red bars and points represent non-phenotypically variable size treatments (NV) of each strain.

2.3.2.3. Other identified effects

Individuals that took longer to emerge achieved heavier adult body weights on average but also exhibited more variation in body weight (Table 2.1 & Table 2A.3). In replicates with larger final population size, males took longer to emerge, females achieved larger adult body weight and there was greater variation in body weight among individuals (Table 2.2, Table 2.3 & Table 2A.4). Populations with more emerged females had larger body and total biomass (Table 2.1, Table 2.2, Table 2A.3, Table 2A.4).

2.4. Discussion

The present study aimed to explore whether phenotypic and genotypic intraspecific variation in *S. oryzae* affected their performance in novel and heterogeneous environments. Theory suggests that intraspecific variation may be important under novel and varying conditions (Wright et al., 2016), and therefore we hypothesised that more genetically variable strains and more phenotypically variable subpopulations would perform better in novel and heterogeneous environments. However, our results revealed no consistent benefits of phenotypic intraspecific variation and only partially supported the expectation of better performance due to increased genetic variation expected in the hybrid strain.

2.4.1. Genotypic variation

As predicted, differences between strains were found in all tested variables. Agashe (2009) also found differences in performance of different strains of *T. castaneum* collected from geographically different sources. However, contrary to our expectation, differences between strains in our study were not always explained by expected

genotypic variation. The hybrid strain TAQ was among the best performing in some cases, but in other instances, its performance was intermediate to that of the other strains (from which TAQ was created). Reduced performance in some cases could be the result of hybrid breakdown, however the results seem to more likely reflect trade-offs in hybrids different performance traits. For example, TAQ had one of the lowest population sizes but also produced the largest individuals on mixed grain, which could reflect a trade-off between quality and quantity of offspring. Insects that produce bigger but fewer offspring may benefit by their offspring being more fecund (Honěk, 1993). On the other hand, having many smaller offspring may be beneficial as each individual offspring requires less food and develops faster (Holloway et al., 1990; Sibly and Calow, 1986). Assessing and comparing more traits, as well as genetic sequence and methylation comparisons between the single and hybrid strains, may help reveal if the performance is more to do with hybrid breakdown or trade-offs in performance traits.

Contrary to our prediction that more genetically variable strains would perform best in novel and heterogenous environments (i.e. mixed grain), TAQ often performed best on the familiar wheat grain, possibly reflecting adaptation to that environment in the parent strains. Agashe (2009) also found larger population sizes in familiar grains in red flour beetles *T. castaneum*. However, increased genetic variation (resulting from allowing interbreeding of distinct strains as done here) led to larger population sizes in red flour beetles, with a stronger benefit in the novel grain environment (at highest level of genetic variation, population sizes in familiar and novel grain were comparable). While we also found that genetic variation led to larger population sizes within the familiar grain, we did not observe larger population sizes in TAQ within novel grains.

2.4.2. Phenotypic variation

Phenotypic variation did not affect most of the variables that we assessed, apart from body weight on novel grains; but this effect was not as predicted for both sexes. Males in phenotypically variable (PV) groups had the largest body masses within mixed grains, as we expected. However, among females, it was the genetically variable but phenotypically similar (TAQ NV groups) individuals that had the largest body masses. The results therefore suggest that phenotypic variation benefits males in novel, heterogenous environments, but offers no beneficial effects for females. It is plausible that phenotypic variation affects males and females differently, as insect species commonly display sex differences in traits such as body weight (see review by Sukhodolskaya, Saveliev and Muhammetnabiev, 2016). While further research is needed to understand these potential differences, our results highlight the importance of considering sex when studying the effects of phenotypic variation.

Although many studies have reported overall positive effects of intraspecific variation on population performance (Forsman and Wennersten, 2016), there are also studies which have also shown that maintaining phenotypic variation can lead to trade-offs (in size at metamorphosis; Lind and Johansson, 2009), and negative effects like outbreeding depression (Edmands, 1999). It is likely that the effects of intraspecific variation are context specific and vary depending on the mechanisms responsible for generating variation as suggested by our study. As mentioned, we found that genetic variation between strains/populations of *S. oryzae* influenced performance, possibly due to differences resulting from local adaptation and gene flow in these geographically distinct strains. However, when we assessed phenotypic variation within populations, we did not find that this variation strongly influence performance. Phenotypic variation may occur due to various mechanisms including genetic differences, phenotypic plasticity (in which

the same genotype expressed different phenotypes depending on the environment; Hallgrímsson and Hall, 2005), and developmental plasticity (built-in molecular mechanisms that can maintain variation; Lea et al., 2017). Depending on the mechanism driving phenotypic variation we may see different benefits and inter-generational effects (i.e., if parents are phenotypically variable how does that influence their offspring?). In our study, variation in offspring body weight of *S. oryzae* at the population level was not clearly associated to variation in parental weight, which suggests body size is fairly plastic and the inter-generational effects we wanted to assess may be difficult to predict.

Future studies should explore longer term consequences and consider whether phenotypic variation in other traits plays a stronger role in performance of *S. oryzae*. In pygmy grasshoppers, *Tetrix subulate*, Forsman et al. (2012) found that the relative frequency of the different colour morphs (which differed in morphology, physiology, behaviour and reproductive life-history), sampled a year after the founder populations were initially released, were very different. Over time, some colour morphs increased in frequency whilst others decreased, suggesting between generation differences, most likely due to selection of particular morphs. Phenotypic effects could therefore be revealed after several generations of selection, and therefore future studies should evaluate effects over multiple generations if possible. The effects of phenotypic variation may also be clearer under natural conditions (Forsman, 2014). While in standardized laboratory conditions we found no strong effects of variation in body weight of *S. oryzae*, studies under more natural conditions may show different patterns. Additionally, further experiments under different levels of resource competition/ environmental stress in our treatments would help indicate whether there is any condition where phenotypic variation within strains is important.

2.4.3. Environmental Variation

All strains and sub-populations performed well on wheat and/or mixed grains for most of the variables we assessed, apart from body size, where all strains performed best on the novel grain maize. Agashe (2009) also found that for less genetically variable populations (comparable to our single strain treatments), the greatest population size was found in the familiar wheat habitat, compared to the novel wheat and corn habitat that was tested. The improved performance on wheat and mixed grains (which included wheat) in our study could therefore be due to local adaptation, whereby alleles linked to feeding on wheat grains are more fixed through selection. Adaptation to wheat is suggested by the more stable body weights (presumably closer to an optimal) we observed in that grain compared to on other grains. The improved performance we saw on wheat and mixed grains could also be due to the wheat environment providing more and/or better-quality resources for larvae to develop compared to the other grains. Bigger grains are likely to have more food available for larva to consume for growth and development before emerging as an adult. Indeed, the smallest body sizes for all strains were found in rice which was the smallest tested grain. The benefits of larger grains have been previously shown in populations of the seed-feeding beetle *Stator limbatus*, which have faster development and higher survival when reared on the larger host seed, *Acacia greggii* (Amarillo-Suárez and Fox, 2006).

2.4.3.1. Potential implications for pest management

We found the number of offspring was lower in novel and mixed grains for all strains likely due to adaption to their familiar grain. This suggests a possible management strategy to reduce the impact of this pest insect that could work in different locations and would involve alternating between growing and storing different grains over time to

regularly create “novel” environments. Alternating may be required on a relatively frequent (e.g., annual) basis to be effective. Agashe et al. (2011) found that ancestrally wheat-bred populations of *T. castaneum* became locally adapted and preferred corn to wheat after only two years. Besides the potential pest control benefits, crop rotation offers other benefits to farmers in the long term such as increasing yield (Berzsenyi et al., 2000). Of course, before any crop rotation strategy is implemented, further research is needed to evaluate the impact of alternating grains on *S. oryzae* populations over several generations.

2.4.4. Conclusions

Overall, we found consistent grain and strain (genetic variation) effects on the performance of the storage pest *S. oryzae*, but not clear effect of phenotypic variation. While much of the recent ecological literature, after initially ignoring within-species variation, has emphasized the importance of intraspecific variation, here we show effects may be context specific and that variation is not always beneficial or influential. Our results have some implications for management of grain storage providing support for grain rotation as a potential tool for pest control. Our study also raises a number of questions, regarding whether phenotypic variation in other traits could influence performance, whether effects may be more noticeable over multiple generations and how variation across sexes could potentially influenced performance differently.

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Appendix

Table 2A.1 Body weight variance (mg) of *S. oryzae* males (M) and females (F) from strains: the hybrid strain TAQ, Africa, Queensland and Trinidad. Strains were further divided into non-variable (NV) and variable (PV) subpopulations.

Phenotypic	**TAQ**		**Africa**		**Queensland**		**Trinidad**	
variation	M	F	M	F	M	F	M	F
NV	2.4 – 2.6	2.5 – 2.7	2.4 – 2.6	2.5 – 2.7	2.4 – 2.6	2.5 – 2.7	2.3 – 2.5	2.3 – 2.5
PV	2.0 – 2.9	2.2 – 3.1	2.1 – 3.0	2.1 – 3.0	2.0 – 2.9	2.1 – 3.0	1.9 – 2.8	1.9 – 2.8

Table 2A.2 All models tested to analyse the effects of phenotypic variation, strain and grain type on weevil offspring performance: (a) body weight; (b) development period; (c) growth rate; (d) population size; (e) body weight variation and (f) total biomass. * indicates tested interactions and models used are highlighted in bold.

Model	Fixed predictors	Random Factors
Individual Level		
Body weight - Females		
BF1	Phenotypic variation + strain + grain type + development period + total population size	Date set up: replicate code
BF2	Phenotypic variation*strain + grain type + development period + total population size	Date set up: replicate code
BF3	Phenotypic variation*grain type + strain + development period + total population size	Date set up: replicate code
BF4	Grain type*strain + phenotypic variation + development period + total population size	Date set up: replicate code
BF5	Phenotypic variation*(grain type +strain) + development period + total population size	Date set up: replicate code
BF6	**Strain*(phenotypic variation + grain type) + development period + total population size**	**Date set up: replicate code**
BF7	Grain type*(phenotypic variation + strain) + development period + total population size	Date set up: replicate code
Body weight - Males		
BM1	Phenotypic variation + strain + grain type + development period + total population size	Date set up: replicate code
BM2	Phenotypic variation*strain + grain type + development period + total population size	Date set up: replicate code
BM3	Phenotypic variation*grain type + strain + development period + total population size	Date set up: replicate code
BM4	Grain type*strain + phenotypic variation + development period + total population size	Date set up: replicate code
BM5	Phenotypic variation*(grain type +strain) + development period + total population size	Date set up: replicate code
BM6	Strain*(phenotypic variation + grain type) + development period + total population size	Date set up: replicate code

BM7	**Grain type*(phenotypic variation + strain) + development period + total population size**	**Date set up: replicate code**
Development period - Females		
DF1	**Phenotypic variation + strain + grain type + offspring body weight + total population size**	**Date set up: replicate code**
DF2	Phenotypic variation*strain + grain type + offspring body weight + total population size	Date set up: replicate code
DF3	Phenotypic variation*grain type + strain + offspring body weight + total population size	Date set up: replicate code
DF4	Grain type*strain + phenotypic variation + offspring body weight + total population size	Date set up: replicate code
DF5	Phenotypic variation*(grain type + strain) + offspring body weight + total population size	Date set up: replicate code
DF6	Strain*(phenotypic variation + grain type) + offspring body weight + total population size	Date set up: replicate code
DF7	Grain type*(phenotypic variation + strain) + offspring body weight + total population size	Date set up: replicate code
Development period - Males		
DM1	**Phenotypic variation + strain + grain type + offspring body weight + total population size**	**Date set up: replicate code**
DM2	Phenotypic variation*strain + grain type + offspring body weight + total population size	Date set up: replicate code
DM3	Phenotypic variation*grain type + strain + offspring body weight + total population size	Date set up: replicate code
DM4	Grain type*strain + phenotypic variation + offspring body weight + total population size	Date set up: replicate code
DM5	Phenotypic variation*(grain type + strain) + offspring body weight + total population size	Date set up: replicate code
DM6	Strain*(phenotypic variation + grain type) + offspring body weight + total population size	Date set up: replicate code
DM7	Grain type*(phenotypic variation + strain) + offspring body weight + total population size	Date set up: replicate code
Growth rate - Females		
GF1	**Phenotypic variation + strain + grain type + total population size**	**Date set up: replicate code**

GF2	Phenotypic variation*strain + grain type + total population size	Date set up: replicate code
GF3	Phenotypic variation*grain type + strain + total population size	Date set up: replicate code
GF4	Grain type*strain + phenotypic variation + total population size	Date set up: replicate code
GF5	Phenotypic variation*(grain type + strain) + total population size	Date set up: replicate code
GF6	Strain*(phenotypic variation + grain type) + total population size	Date set up: replicate code
GF7	Grain type*(phenotypic variation + strain) + total population size	Date set up: replicate code
Growth rate – Males		
GM1	**Phenotypic variation + strain + grain type + total population size**	**Date set up: replicate code**
GM2	Phenotypic variation*strain + grain type + total population size	Date set up: replicate code
GM3	Phenotypic variation*grain type + strain + total population size	Date set up: replicate code
GM4	Grain type*strain + phenotypic variation + total population size	Date set up: replicate code
GM5	Phenotypic variation*(grain type + strain) + total population size	Date set up: replicate code
GM6	Strain*(phenotypic variation + grain type) + total population size	Date set up: replicate code
GM7	Grain type*(phenotypic variation + strain) + total population size	Date set up: replicate code
Population Level		
Total population size		
P1	Phenotypic variation + strain + grain type	Date set up
P2	Phenotypic variation*strain + grain type	Date set up
P3	Phenotypic variation*grain type + strain	Date set up
P4	**Grain type*strain + phenotypic variation**	**Date set up**
P5	Phenotypic variation*(grain type + strain)	Date set up
P6	Strain*(phenotypic variation + grain type)	Date set up

P7	Grain type*(phenotypic variation + strain)	Date set up
Body weight variation		
V1	**Phenotypic variation + strain + grain type + mean development period + total population size + percentage of females**	**Date set up**
V2	Phenotypic variation*strain + grain type + mean development period + total population size + percentage of females	Date set up
V3	Phenotypic variation*grain type + strain + mean development period + total population size + percentage of females	Date set up
V4	Grain type*strain + phenotypic variation + mean development period + total population size + percentage of females	Date set up
V5	Phenotypic variation*(grain type + strain) + mean development period + total population size + percentage of females	Date set up
V6	Strain*(phenotypic variation + grain type) + mean development period + total population size + percentage of females	Date set up
V7	Grain type*(phenotypic variation + strain) + mean development period + total population size + percentage of females	Date set up
Total biomass		
T1	Phenotypic variation + strain + grain type + mean development period + percentage of females	Date set up
T2	Phenotypic variation*strain + grain type + mean development period + percentage of females	Date set up
T3	Phenotypic variation*grain type + strain + mean development period + percentage of females	Date set up

T4	**Grain type*strain + phenotypic variation + mean development period + percentage of females**	**Date set up**
T5	Phenotypic variation*(grain type + strain) + mean development period + percentage of females	Date set up
T6	Strain*(phenotypic variation + grain type) + mean development period + percentage of females	Date set up
T7	Grain type*(phenotypic variation + strain) + mean development period + percentage of females	Date set up

Table 2A.3 Differences of least squares means and confidence intervals for models predicting *S. oryzae* individual responses of body weight as a function of phenotypic variation (PV), *S. oryzae* strain (hybrid strain TAQ, Africa, Queensland and Trinidad), the grain type (wheat, rice, maize and mixture of all three grains), sex, development period, offspring body weight. We report best parameter estimates (β), standard error (SE), degrees of freedom (DF), t- value, their 95% confidence interval (CI), and P-value. The colon separating variable names indicates interaction terms. Significant P-values are highlighted in bold.

Comparison	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Body weight – Females							
Phenotypic variation							
NV – PV	0.005	0.009	220.180	0.537	-0.014	0.024	0.592
Strain							
TAQ – Africa	-0.011	0.014	291.092	-0.753	-0.038	0.017	0.452
TAQ – Queensland	**-0.043**	**0.014**	**292.275**	**-3.106**	**-0.070**	**-0.016**	**0.002**
TAQ – Trinidad	**0.061**	**0.014**	**333.322**	**4.305**	**0.033**	**0.089**	**<0.0001**
Africa – Queensland	**-0.032**	**0.014**	**292.273**	**-2.310**	**-0.060**	**-0.005**	**0.022**
Africa – Trinidad	**0.072**	**0.014**	**329.997**	**5.015**	**0.044**	**0.100**	**<0.0001**
Queensland – Trinidad	**0.104**	**0.014**	**331.770**	**7.312**	**0.076**	**0.132**	**<0.0001**
Grain							
Wheat – Rice	**0.171**	**0.021**	**226.354**	**8.080**	**0.129**	**0.213**	**<0.0001**

Comparison	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Wheat – Maize	**-0.224**	**0.024**	**263.661**	**-9.410**	**-0.271**	**-0.177**	**<0.0001**
Wheat – Mixture	-0.007	0.015	187.519	-0.474	-0.038	0.023	0.636
Rice – Maize	**-0.395**	**0.016**	**459.027**	**-24.274**	**-0.427**	**-0.363**	**<0.0001**
Rice – Mixture	**-0.179**	**0.015**	**281.267**	**-11.582**	**-0.209**	**-0.148**	**<0.0001**
Maize – Mixture	**0.217**	**0.017**	**346.069**	**12.519**	**0.183**	**0.251**	**<0.0001**
Within NV, compare strain							
TAQ:NV – Africa:NV	0.016	0.019	264.708	0.802	-0.023	0.054	0.423
TAQ:NV – Queensland:NV	-0.007	0.019	254.701	-0.367	-0.044	0.030	0.714
TAQ:NV – Trinidad:NV	**0.094**	**0.019**	**274.414**	**4.874**	**0.056**	**0.132**	**<0.0001**
Africa:NV – Queensland:NV	-0.023	0.019	259.480	-1.171	-0.060	0.015	0.243
Africa:NV – Trinidad:NV	**0.079**	**0.020**	**278.002**	**4.021**	**0.040**	**0.117**	**<0.0001**
Queensland:NV – Trinidad:NV	**0.101**	**0.019**	**267.005**	**5.291**	**0.064**	**0.139**	**<0.0001**
Within PV, compare strain							
TAQ:PV – Africa:PV	-0.037	0.019	242.517	-1.914	-0.074	0.001	0.057
TAQ:PV – Queensland:PV	**-0.079**	**0.019**	**246.605**	**-4.108**	**-0.116**	**-0.041**	**<0.0001**
TAQ:PV – Trinidad:PV	0.028	0.020	269.390	1.441	-0.010	0.067	0.151
Africa:PV – Queensland:PV	**-0.042**	**0.019**	**249.629**	**-2.163**	**-0.080**	**-0.004**	**0.031**
Africa:PV – Trinidad:PV	**0.065**	**0.020**	**269.121**	**3.283**	**0.026**	**0.104**	**0.001**
Queensland:PV – Trinidad:PV	**0.107**	**0.020**	**275.898**	**5.389**	**0.068**	**0.146**	**<0.0001**

Comparison	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Within strain, compare phenotypic variation							
TAQ:NV – TAQ:PV	**0.053**	**0.019**	**213.594**	**2.834**	**0.016**	**0.089**	**0.005**
Africa:NV – Africa:PV	0.000	0.019	225.462	0.022	-0.037	0.038	0.983
Queensland:NV – Queensland:PV	-0.019	0.019	216.222	-1.026	-0.056	0.018	0.306
Trinidad:NV – Trinidad:PV	-0.014	0.019	224.450	-0.714	-0.051	0.024	0.476
Within strain, compare grains							
TAQ							
TAQ:Wheat – TAQ:Rice	**0.177**	**0.032**	**216.420**	**5.459**	**0.113**	**0.241**	**<0.0001**
TAQ:Wheat – TAQ:Maize	**-0.227**	**0.034**	**252.004**	**-6.608**	**-0.295**	**-0.159**	**<0.0001**
TAQ:Wheat – TAQ:Mixture	-0.001	0.027	175.279	-0.021	-0.054	0.053	0.983
TAQ:Rice – TAQ:Maize	**-0.404**	**0.031**	**423.350**	**-13.024**	**-0.465**	**-0.343**	**<0.0001**
TAQ:Rice – TAQ:Mixture	**-0.178**	**0.028**	**290.363**	**-6.287**	**-0.234**	**-0.122**	**<0.0001**
TAQ:Maize – TAQ:Mixture	**0.226**	**0.029**	**340.551**	**7.732**	**0.169**	**0.284**	**<0.0001**
Africa							
Africa:Wheat – Africa:Rice	**0.227**	**0.030**	**234.046**	**7.512**	**0.168**	**0.287**	**<0.0001**
Africa:Wheat – Africa:Maize	**-0.192**	**0.033**	**301.175**	**-5.823**	**-0.257**	**-0.127**	**<0.0001**
Africa:Wheat – Africa:Mixture	0.019	0.026	189.595	0.736	-0.032	0.069	0.463

Comparison	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Africa:Rice – Africa:Maize	**-0.420**	**0.032**	**403.563**	**-13.049**	**-0.483**	**-0.356**	**<0.0001**
Africa:Rice – Africa:Mixture	**-0.209**	**0.028**	**266.770**	**-7.331**	**-0.265**	**-0.153**	**<0.0001**
Africa:Maize – Africa:Mixture	**0.211**	**0.030**	**351.247**	**6.987**	**0.152**	**0.270**	**<0.0001**
Queensland							
Queensland:Wheat – Queensland:Rice	**0.190**	**0.032**	**234.947**	**6.031**	**0.128**	**0.252**	**<0.0001**
Queensland:Wheat – Queensland:Maize	**-0.168**	**0.034**	**274.481**	**-5.012**	**-0.234**	**-0.102**	**<0.0001**
Queensland:Wheat – Queensland:Mixture	-0.037	0.026	175.450	-1.453	-0.088	0.013	0.148
Queensland:Rice – Queensland:Maize	**-0.358**	**0.032**	**422.015**	**-11.319**	**-0.421**	**-0.296**	**<0.0001**
Queensland:Rice – Queensland:Mixture	**-0.227**	**0.029**	**285.753**	**-7.952**	**-0.284**	**-0.171**	**<0.0001**
Queensland:Maize – Queensland:Mixture	**0.131**	**0.030**	**344.568**	**4.364**	**0.072**	**0.190**	**<0.0001**
Trinidad							
Trinidad:Wheat – Trinidad:Rice	**0.090**	**0.032**	**275.686**	**2.825**	**0.027**	**0.153**	**0.005**
Trinidad:Wheat – Trinidad:Maize	**-0.309**	**0.036**	**361.515**	**-8.659**	**-0.380**	**-0.239**	**<0.0001**

Comparison	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Trinidad:Wheat – Trinidad:Mixture	-0.010	0.024	166.848	-0.435	-0.058	0.037	0.664
Trinidad:Rice – Trinidad:Maize	**-0.399**	**0.034**	**600.336**	**-11.760**	**-0.466**	**-0.333**	**<0.0001**
Trinidad:Rice – Trinidad:Mixture	**-0.100**	**0.030**	**310.834**	**-3.385**	**-0.159**	**-0.042**	**0.001**
Trinidad:Maize – Trinidad:Mixture	**0.299**	**0.033**	**422.732**	**8.980**	**0.234**	**0.365**	**<0.0001**
Within grains, compare strain							
Wheat							
TAQ:Wheat – Africa:Wheat	-0.037	0.023	135.582	-1.612	-0.081	0.008	0.109
TAQ:Wheat – Queensland:Wheat	**-0.052**	**0.022**	**130.480**	**-2.358**	**-0.095**	**-0.008**	**0.020**
TAQ:Wheat – Trinidad:Wheat	**0.106**	**0.022**	**134.598**	**4.774**	**0.062**	**0.150**	**<0.0001**
Africa:Wheat – Queensland:Wheat	-0.015	0.023	141.160	-0.666	-0.060	0.030	0.506
Africa:Wheat – Trinidad:Wheat	**0.143**	**0.022**	**145.823**	**6.352**	**0.098**	**0.187**	**<0.0001**
Queensland:Wheat – Trinidad:Wheat	**0.158**	**0.022**	**139.316**	**7.141**	**0.114**	**0.201**	**<0.0001**
Rice							
TAQ:Rice – Africa:Rice	0.014	0.031	338.635	0.438	-0.047	0.074	0.661
TAQ:Rice – Queensland:Rice	-0.039	0.031	363.555	-1.263	-0.099	0.022	0.208
TAQ:Rice – Trinidad:Rice	0.019	0.031	413.160	0.605	-0.042	0.080	0.545
Africa:Rice – Queensland:Rice	-0.052	0.031	335.960	-1.688	-0.113	0.009	0.092
Africa:Rice – Trinidad:Rice	0.005	0.031	377.985	0.168	-0.056	0.067	0.866

Comparison	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Queensland:Rice – Trinidad:Rice	0.058	0.031	404.852	1.850	-0.004	0.119	0.065
Maize							
TAQ:Maize – Africa:Maize	-0.002	0.032	499.306	-0.054	-0.065	0.061	0.957
TAQ:Maize – Queensland:Maize	0.007	0.032	489.472	0.228	-0.055	0.070	0.820
TAQ:Maize – Trinidad:Maize	0.024	0.034	618.786	0.697	-0.043	0.091	0.486
Africa:Maize – Queensland:Maize	0.009	0.033	506.723	0.276	-0.055	0.073	0.783
Africa:Maize – Trinidad:Maize	0.025	0.035	635.120	0.734	-0.043	0.093	0.463
Queensland:Maize – Trinidad:Maize	0.016	0.034	622.754	0.477	-0.051	0.084	0.633
Mixture							
TAQ:Mixture – Africa:Mixture	-0.017	0.025	217.934	-0.690	-0.066	0.032	0.491
TAQ:Mixture – Queensland:Mixture	**-0.088**	**0.025**	**211.667**	**-3.584**	**-0.137**	**-0.040**	**<0.0001**
TAQ:Mixture – Trinidad:Mixture	**0.096**	**0.025**	**206.495**	**3.915**	**0.048**	**0.145**	**0.000**
Africa:Mixture – Queensland:Mixture	**-0.071**	**0.025**	**211.698**	**-2.870**	**-0.120**	**-0.022**	**0.005**
Africa:Mixture – Trinidad:Mixture	**0.114**	**0.025**	**202.028**	**4.616**	**0.065**	**0.162**	**<0.0001**
Queensland:Mixture – Trinidad:Mixture	**0.185**	**0.024**	**199.591**	**7.581**	**0.137**	**0.233**	**<0.0001**
Body weight – Males							

Comparison	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Phenotypic variation							
NV – PV	**-0.019**	**0.009**	**328.260**	**-2.069**	**-0.037**	**-0.001**	**0.039**
Strain							
TAQ – Africa	-0.006	0.013	328.425	-0.469	-0.032	0.020	0.640
TAQ – Queensland	**-0.037**	**0.013**	**302.990**	**-2.920**	**-0.062**	**-0.012**	**0.004**
TAQ – Trinidad	**0.065**	**0.013**	**365.685**	**4.895**	**0.039**	**0.092**	**<0.0001**
Africa – Queensland	**-0.031**	**0.013**	**306.457**	**-2.404**	**-0.056**	**-0.006**	**0.017**
Africa – Trinidad	**0.072**	**0.013**	**362.186**	**5.372**	**0.045**	**0.098**	**<0.0001**
Queensland – Trinidad	**0.102**	**0.013**	**340.207**	**7.827**	**0.077**	**0.128**	**<0.0001**
Grain							
Wheat – Rice	**0.179**	**0.018**	**223.914**	**9.948**	**0.143**	**0.214**	**<0.0001**
Wheat – Maize	**-0.172**	**0.021**	**279.664**	**-8.319**	**-0.213**	**-0.131**	**<0.0001**
Wheat – Mixture	0.006	0.014	183.081	0.422	-0.021	0.033	0.674
Rice – Maize	**-0.351**	**0.015**	**501.041**	**-23.126**	**-0.380**	**-0.321**	**<0.0001**
Rice – Mixture	**-0.173**	**0.014**	**310.083**	**-12.430**	**-0.200**	**-0.145**	**<0.0001**
Maize – Mixture	**0.178**	**0.016**	**403.521**	**11.295**	**0.147**	**0.209**	**<0.0001**
Within strain, compare grain							
TAQ							

Comparison	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Wheat:TAQ – Rice:TAQ	**0.172**	**0.027**	**217.848**	**6.260**	**0.118**	**0.226**	**<0.0001**
Wheat:TAQ – Maize:TAQ	**-0.160**	**0.031**	**297.453**	**-5.083**	**-0.222**	**-0.098**	**<0.0001**
Wheat:TAQ – Mixture:TAQ	0.030	0.023	172.411	1.302	-0.016	0.077	0.195
Rice:TAQ – Maize:TAQ	**-0.331**	**0.030**	**474.793**	**-11.203**	**-0.390**	**-0.273**	**<0.0001**
Rice:TAQ – Mixture:TAQ	**-0.141**	**0.026**	**285.524**	**-5.519**	**-0.191**	**-0.091**	**<0.0001**
Maize:TAQ – Mixture:TAQ	**0.190**	**0.029**	**403.162**	**6.649**	**0.134**	**0.247**	**<0.0001**
Africa							
Wheat:Africa – Rice:Africa	**0.206**	**0.027**	**244.764**	**7.601**	**0.153**	**0.260**	**<0.0001**
Wheat:Africa – Maize:Africa	**-0.179**	**0.030**	**330.644**	**-5.981**	**-0.237**	**-0.120**	**<0.0001**
Wheat:Africa – Mixture:Africa	-0.002	0.023	188.065	-0.108	-0.048	0.043	0.914
Rice:Africa – Maize:Africa	**-0.385**	**0.030**	**466.172**	**-12.851**	**-0.444**	**-0.326**	**<0.0001**
Rice:Africa – Mixture:Africa	**-0.209**	**0.026**	**301.824**	**-7.887**	**-0.261**	**-0.157**	**<0.0001**
Maize:Africa – Mixture:Africa	**0.176**	**0.028**	**418.513**	**6.193**	**0.120**	**0.232**	**<0.0001**
Queensland							
Wheat:Queensland – Rice:Queensland	**0.216**	**0.027**	**224.521**	**7.910**	**0.162**	**0.270**	**<0.0001**
Wheat:Queensland – Maize:Queensland	**-0.148**	**0.029**	**264.822**	**-5.185**	**-0.204**	**-0.092**	**<0.0001**

Comparison	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Wheat:Queensland – Mixture:Queensland	-0.017	0.023	182.157	-0.748	-0.063	0.029	0.456
Rice:Queensland – Maize:Queensland	**-0.364**	**0.028**	**402.403**	**-12.978**	**-0.419**	**-0.309**	**<0.0001**
Rice:Queensland – Mixture:Queensland	**-0.233**	**0.026**	**295.277**	**-9.036**	**-0.284**	**-0.183**	**<0.0001**
Maize:Queensland – Mixture:Queensland	**0.131**	**0.026**	**347.024**	**4.935**	**0.079**	**0.183**	**<0.0001**
Trinidad							
Wheat:Trinidad – Rice:Trinidad	**0.120**	**0.030**	**286.244**	**4.052**	**0.062**	**0.179**	**<0.0001**
Wheat:Trinidad – Maize:Trinidad	**-0.202**	**0.032**	**373.130**	**-6.240**	**-0.265**	**-0.138**	**<0.0001**
Wheat:Trinidad – Mixture:Trinidad	0.013	0.023	181.715	0.549	-0.033	0.058	0.583
Rice:Trinidad – Maize:Trinidad	**-0.322**	**0.032**	**654.516**	**-10.125**	**-0.384**	**-0.260**	**<0.0001**
Rice:Trinidad – Mixture:Trinidad	**-0.108**	**0.028**	**360.913**	**-3.878**	**-0.162**	**-0.053**	**<0.0001**
Maize:Trinidad – Mixture:Trinidad	**0.214**	**0.030**	**476.741**	**7.192**	**0.156**	**0.273**	**<0.0001**
Within grain, compare phenotypic variation							
Wheat:NV – Wheat:PV	0.014	0.014	133.429	1.008	-0.014	0.042	0.315
Rice:NV – Rice:PV	-0.029	0.020	391.026	-1.454	-0.069	0.010	0.147

Comparison	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Maize:NV – Maize:PV	-0.010	0.022	598.853	-0.455	-0.053	0.033	0.649
Mixture:NV – Mixture:PV	**-0.051**	**0.016**	**232.060**	**-3.112**	**-0.083**	**-0.019**	**0.002**
Within NV, compare grain							
Wheat:NV – Rice:NV	**0.200**	**0.022**	**259.053**	**8.922**	**0.156**	**0.245**	**<0.0001**
Wheat:NV – Maize:NV	**-0.160**	**0.024**	**301.301**	**-6.572**	**-0.208**	**-0.112**	**<0.0001**
Wheat:NV – Mixture:NV	**0.038**	**0.017**	**181.230**	**2.232**	**0.004**	**0.072**	**0.027**
Rice:NV – Maize:NV	**-0.360**	**0.022**	**499.253**	**-16.740**	**-0.403**	**-0.318**	**<0.0001**
Rice:NV – Mixture:NV	**-0.162**	**0.020**	**324.979**	**-8.287**	**-0.201**	**-0.124**	**<0.0001**
Maize:NV – Mixture:NV	**0.198**	**0.021**	**392.874**	**9.478**	**0.157**	**0.239**	**<0.0001**
Within PV, compare grain							
Wheat:PV – Rice:PV	**0.157**	**0.021**	**210.991**	**7.436**	**0.115**	**0.198**	**<0.0001**
Wheat:PV – Maize:PV	**-0.184**	**0.024**	**290.534**	**-7.528**	**-0.232**	**-0.136**	**<0.0001**
Wheat:PV – Mixture:PV	-0.027	0.018	181.896	-1.501	-0.062	0.008	0.135
Rice:PV – Maize:PV	**-0.341**	**0.021**	**489.766**	**-16.283**	**-0.382**	**-0.300**	**<0.0001**
Rice:PV – Mixture:PV	**-0.183**	**0.019**	**299.364**	**-9.905**	**-0.220**	**-0.147**	**<0.0001**
Maize:PV – Mixture:PV	**0.157**	**0.021**	**417.037**	**7.622**	**0.117**	**0.198**	**<0.0001**

Comparison	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Within grain, compare strain							
Wheat							
Wheat:TAQ – Wheat:Africa	-0.002	0.020	137.141	-0.093	-0.042	0.038	0.926
Wheat:TAQ – Wheat:Queensland	**-0.039**	**0.020**	**128.140**	**-1.986**	**-0.078**	**0.000**	**0.049**
Wheat:TAQ – Wheat:Trinidad	**0.093**	**0.020**	**130.440**	**4.678**	**0.054**	**0.132**	**<0.0001**
Wheat:Africa – Wheat:Queensland	-0.037	0.020	138.327	-1.838	-0.077	0.003	0.068
Wheat:Africa – Wheat:Trinidad	**0.095**	**0.020**	**141.406**	**4.666**	**0.055**	**0.135**	**<0.0001**
Wheat:Queensland – Wheat:Trinidad	**0.132**	**0.020**	**133.002**	**6.631**	**0.093**	**0.171**	**<0.0001**
Rice							
Rice:TAQ – Rice:Africa	0.033	0.028	347.169	1.176	-0.022	0.088	0.240
Rice:TAQ – Rice:Queensland	0.005	0.028	349.476	0.196	-0.049	0.060	0.845
Rice:TAQ – Rice:Trinidad	0.042	0.029	429.601	1.439	-0.015	0.099	0.151
Rice:Africa – Rice:Queensland	-0.027	0.028	353.657	-0.974	-0.083	0.028	0.331
Rice:Africa – Rice:Trinidad	0.009	0.030	426.192	0.306	-0.049	0.067	0.760
Rice:Queensland – Rice:Trinidad	0.037	0.029	429.034	1.246	-0.021	0.094	0.213
Maize							
Maize:TAQ – Maize:Africa	-0.021	0.031	628.567	-0.664	-0.082	0.041	0.507
Maize:TAQ – Maize:Queensland	-0.027	0.030	535.808	-0.914	-0.086	0.031	0.361

Comparison	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Maize:TAQ – Maize:Trinidad	0.051	0.032	717.218	1.598	-0.012	0.114	0.111
Maize:Africa – Maize:Queensland	-0.007	0.030	526.914	-0.222	-0.065	0.051	0.825
Maize:Africa – Maize:Trinidad	**0.072**	**0.032**	**707.532**	**2.258**	**0.009**	**0.135**	**0.024**
Maize:Queensland – Maize:Trinidad	**0.079**	**0.031**	**613.306**	**2.567**	**0.018**	**0.139**	**0.010**
Mixture							
Mixture:TAQ – Mixture:Africa	-0.035	0.023	231.271	-1.499	-0.081	0.011	0.135
Mixture:TAQ – Mixture:Queensland	**-0.087**	**0.023**	**227.615**	**-3.798**	**-0.132**	**-0.042**	**<0.0001**
Mixture:TAQ – Mixture:Trinidad	**0.075**	**0.023**	**227.913**	**3.282**	**0.030**	**0.120**	**0.001**
Mixture:Africa – Mixture:Queensland	**-0.052**	**0.023**	**236.716**	**-2.247**	**-0.098**	**-0.006**	**0.026**
Mixture:Africa – Mixture:Trinidad	**0.110**	**0.023**	**232.796**	**4.755**	**0.064**	**0.156**	**<0.0001**
Mixture:Queensland – Mixture:Trinidad	**0.162**	**0.023**	**233.430**	**7.078**	**0.117**	**0.207**	**<0.0001**

Table 2A.4 Differences of least squares means and confidence intervals for models predicting *S. oryzae* individual responses of development period as a function of phenotypic variation (PV), *S. oryzae* strain (hybrid strain TAQ, Africa, Queensland and Trinidad), the grain type (wheat, rice, maize and mixture of all three grains), sex, development period, offspring body weight. We report best parameter estimates (β), standard error (SE), degrees of freedom (DF), t- value, their 95% confidence interval (CI), and P-value. The colon separating variable names indicates interaction terms. Significant P-values are highlighted in bold.

Variable	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Development period – Females							
Phenotypic variation							
NV – PV	0.086	0.206	246.851	0.419	-0.319	0.492	0.676
Strain							
TAQ – Africa	**-1.139**	**0.292**	**245.264**	**-3.907**	**-1.714**	**-0.565**	**<0.0001**
TAQ – Queensland	-0.474	0.288	242.399	-1.645	-1.041	0.094	0.101
TAQ – Trinidad	**-1.639**	**0.291**	**250.217**	**-5.628**	**-2.213**	**-1.066**	**<0.0001**
Africa – Queensland	**0.666**	**0.292**	**245.663**	**2.279**	**0.09**	**1.241**	**0.024**
Africa – Trinidad	-0.5	0.295	259.535	-1.695	-1.081	0.081	0.091
Queensland – Trinidad	**-1.165**	**0.294**	**258.134**	**-3.969**	**-1.744**	**-0.587**	**<0.0001**
Grain							
Wheat – Rice	**-3.839**	**0.451**	**241.197**	**-8.504**	**-4.729**	**-2.95**	**<0.0001**

Variable	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Wheat – Maize	**-3.743**	**0.502**	**274.811**	**-7.454**	**-4.732**	**-2.755**	**<0.0001**
Wheat – Mixture	-0.556	0.336	210.247	-1.653	-1.218	0.107	0.100
Rice – Maize	0.096	0.368	544.137	0.261	-0.627	0.819	0.794
Rice – Mixture	**3.284**	**0.331**	**287.365**	**9.917**	**2.632**	**3.936**	**<0.0001**
Maize – Mixture	**3.188**	**0.367**	**339.881**	**8.684**	**2.466**	**3.91**	**<0.0001**
Development period – Males							
Phenotypic variation							
NV – PV	0.317	0.218	266.493	1.456	-0.112	0.747	0.147
Strain							
TAQ – Africa	**-1.547**	**0.311**	**266.415**	**-4.981**	**-2.159**	**-0.936**	**<0.0001**
TAQ – Queensland	**-0.796**	**0.305**	**259.544**	**-2.614**	**-1.396**	**-0.196**	**0.009**
TAQ – Trinidad	**-1.644**	**0.312**	**273.719**	**-5.277**	**-2.258**	**-1.031**	**<0.0001**
Africa – Queensland	**0.751**	**0.309**	**262.222**	**2.433**	**0.143**	**1.359**	**0.016**
Africa – Trinidad	-0.097	0.313	280.537	-0.310	-0.714	0.520	0.757
Queensland – Trinidad	**-0.848**	**0.311**	**275.396**	**-2.727**	**-1.461**	**-0.236**	**0.007**
Grain							
Wheat – Rice	**-4.166**	**0.449**	**241.156**	**-9.274**	**-5.051**	**-3.281**	**<0.0001**
Wheat – Maize	**-4.954**	**0.500**	**269.293**	**-9.903**	**-5.939**	**-3.969**	**<0.0001**

Variable	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Wheat – Mixture	**-0.977**	**0.356**	**221.697**	**-2.745**	**-1.679**	**-0.276**	**0.007**
Rice – Maize	**-0.788**	**0.371**	**498.272**	**-2.120**	**-1.517**	**-0.058**	**0.034**
Rice – Mixture	**3.189**	**0.339**	**297.280**	**9.420**	**2.523**	**3.855**	**<0.0001**
Maize – Mixture	**3.977**	**0.369**	**334.611**	**10.769**	**3.250**	**4.703**	**<0.0001**

Table 2A.5 Differences of least squares means and confidence intervals for models predicting *S. oryzae* individual responses of growth rate as a function of phenotypic variation (PV), *S. oryzae* strain (hybrid strain TAQ, Africa, Queensland and Trinidad), the grain type (wheat, rice, maize and mixture of all three grains), sex, development period, offspring body weight. We report best parameter estimates (β), standard error (SE), degrees of freedom (DF), t- value, their 95% confidence interval (CI), and P-value. The colon separating variable names indicates interaction terms. Significant P-values are highlighted in bold.

Variable	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Growth rate – Females							
Phenotypic variation							
NV – PV	0.000	0.000	250.815	-0.465	-0.001	0.001	0.642
Strain							
TAQ – Africa	**0.002**	**0.001**	**248.996**	**4.612**	**0.001**	**0.003**	**<0.0001**
TAQ – Queensland	0.001	0.000	244.469	1.162	0.000	0.002	0.246
TAQ – Trinidad	**0.004**	**0.000**	**249.898**	**7.455**	**0.003**	**0.005**	**<0.0001**
Africa – Queensland	**-0.002**	**0.001**	**249.470**	**-3.463**	**-0.003**	**-0.001**	**0.001**
Africa – Trinidad	**0.001**	**0.001**	**256.599**	**2.794**	**0.000**	**0.002**	**0.006**
Queensland – Trinidad	**0.003**	**0.000**	**250.459**	**6.298**	**0.002**	**0.004**	**<0.0001**
Grain							
Wheat – Rice	**0.008**	**0.001**	**237.624**	**10.991**	**0.007**	**0.010**	**<0.0001**

Variable	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Wheat – Maize	**0.005**	**0.001**	**268.706**	**5.516**	**0.003**	**0.006**	**<0.0001**
Wheat – Mixture	0.001	0.001	213.138	1.320	0.000	0.002	0.188
Rice – Maize	**-0.004**	**0.001**	**440.136**	**-6.380**	**-0.005**	**-0.003**	**<0.0001**
Rice – Mixture	**-0.008**	**0.001**	**277.579**	**-13.723**	**-0.009**	**-0.007**	**<0.0001**
Maize – Mixture	**-0.004**	**0.001**	**323.964**	**-6.385**	**-0.005**	**-0.003**	**<0.0001**
Growth rate – Males							
Phenotypic variation							
NV – PV	-0.001	0.000	267.501	-1.935	-0.001	0.000	0.054
Strain							
TAQ – Africa	**0.003**	**0.001**	**267.723**	**5.296**	**0.002**	**0.004**	**<0.0001**
TAQ – Queensland	**0.001**	**0.001**	**259.159**	**2.001**	**0.000**	**0.002**	**0.046**
TAQ – Trinidad	**0.004**	**0.001**	**270.931**	**6.986**	**0.003**	**0.005**	**<0.0001**
Africa – Queensland	**-0.002**	**0.001**	**263.327**	**-3.363**	**-0.003**	**-0.001**	**0.001**
Africa – Trinidad	0.001	0.001	277.964	1.685	0.000	0.002	0.093
Queensland – Trinidad	**0.003**	**0.001**	**267.646**	**5.064**	**0.002**	**0.004**	**<0.0001**
Grain							
Wheat – Rice	**0.009**	**0.001**	**232.870**	**12.200**	**0.008**	**0.011**	**<0.0001**
Wheat – Maize	**0.006**	**0.001**	**264.627**	**7.487**	**0.005**	**0.008**	**<0.0001**

Variable	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Wheat – Mixture	**0.001**	**0.001**	**221.198**	**2.364**	**0.000**	**0.003**	**0.019**
Rice – Maize	**-0.003**	**0.001**	**419.188**	**-4.825**	**-0.004**	**-0.002**	**<0.0001**
Rice – Mixture	**-0.008**	**0.001**	**285.534**	**-13.705**	**-0.009**	**-0.007**	**<0.0001**
Maize – Mixture	**-0.005**	**0.001**	**325.570**	**-7.882**	**-0.006**	**-0.004**	**<0.0001**

Table 2A.6 Least squares means and confidence intervals for models predicting *S. oryzae* population of total population size responses of growth rate as a function of phenotypic variation (PV), *S. oryzae* strain (hybrid strain TAQ, Africa, Queensland and Trinidad), the grain type (wheat, rice, maize and mixture of all three grains), sex, development period, offspring body weight. We report best parameter estimates (β), standard error (SE), degrees of freedom (DF), Z-ratio and P-value. The colon separating variable names indicates interaction terms. Significant P-values are highlighted in bold.

Variable	β	SE	DF	Z-ratio	*P*-value
Phenotypic variation					
NV – PV	-0.025	0.027	Inf	-0.911	0.362
Strain					
TAQ – Africa	0.085	0.044	Inf	1.917	0.055
TAQ – Queensland	-0.014	0.043	Inf	-0.330	0.742
TAQ – Trinidad	0.072	0.046	Inf	1.551	0.121
Africa – Queensland	**-0.099**	**0.044**	**Inf**	**-2.256**	**0.024**
Africa – Trinidad	-0.013	0.047	Inf	-0.269	0.788
Queensland – Trinidad	0.086	0.046	Inf	1.868	0.062
Grain					
Wheat – Rice	**1.159**	**0.041**	**Inf**	**28.463**	**<0.0001**
Wheat – Maize	**1.449**	**0.046**	**Inf**	**31.794**	**<0.0001**
Wheat – Mixture	**0.523**	**0.032**	**Inf**	**16.146**	**<0.0001**
Rice – Maize	**0.290**	**0.054**	**Inf**	**5.332**	**<0.0001**

Variable	β	SE	DF	Z-ratio	*P*-value
Rice – Mixture	**-0.636**	**0.044**	**Inf**	**-14.454**	**<0.0001**
Maize – Mixture	**-0.926**	**0.049**	**Inf**	**-19.084**	**<0.0001**
Within strain, compare grain					
TAQ					
Wheat:TAQ – Rice:TAQ	**1.217**	**0.078**	**Inf**	**15.646**	**<0.0001**
Wheat:TAQ – Maize:TAQ	**1.527**	**0.088**	**Inf**	**17.347**	**<0.0001**
Wheat:TAQ – Mixture:TAQ	**0.673**	**0.064**	**Inf**	**10.515**	**<0.0001**
Rice:TAQ – Maize:TAQ	**0.310**	**0.105**	**Inf**	**2.948**	**0.003**
Rice:TAQ – Mixture:TAQ	**-0.545**	**0.086**	**Inf**	**-6.342**	**<0.0001**
Maize:TAQ – Mixture:TAQ	**-0.855**	**0.095**	**Inf**	**-8.969**	**<0.0001**
Africa					
Wheat:Africa – Rice:Africa	**1.047**	**0.081**	**Inf**	**12.862**	**<0.0001**
Wheat:Africa – Maize:Africa	**1.348**	**0.091**	**Inf**	**14.754**	**<0.0001**
Wheat:Africa – Mixture:Africa	**0.487**	**0.067**	**Inf**	**7.243**	**<0.0001**
Rice:Africa – Maize:Africa	**0.301**	**0.107**	**Inf**	**2.803**	**0.005**
Rice:Africa – Mixture:Africa	**-0.560**	**0.088**	**Inf**	**-6.377**	**<0.0001**
Maize:Africa – Mixture:Africa	**-0.861**	**0.097**	**Inf**	**-8.865**	**<0.0001**
Queensland					
Wheat:Queensland – Rice:Queensland	**1.129**	**0.080**	**Inf**	**14.129**	**<0.0001**

Variable	β	SE	DF	Z-ratio	*P*-value
Wheat:Queensland – Maize:Queensland	**1.322**	**0.085**	**Inf**	**15.631**	**<0.0001**
Wheat:Queensland – Mixture:Queensland	**0.569**	**0.065**	**Inf**	**8.812**	**<0.0001**
Rice:Queensland – Maize:Queensland	**0.193**	**0.103**	**Inf**	**1.883**	**0.060**
Rice:Queensland – Mixture:Queensland	**-0.560**	**0.087**	**Inf**	**-6.453**	**<0.0001**
Maize:Queensland – Mixture:Queensland	**-0.753**	**0.091**	**Inf**	**-8.266**	**<0.0001**
Trinidad					
Wheat:Trinidad – Rice:Trinidad	**1.244**	**0.087**	**Inf**	**14.381**	**<0.0001**
Wheat:Trinidad – Maize:Trinidad	**1.600**	**0.100**	**Inf**	**16.004**	**<0.0001**
Wheat:Trinidad – Mixture:Trinidad	**0.366**	**0.064**	**Inf**	**5.752**	**<0.0001**
Rice:Trinidad – Maize:Trinidad	**0.357**	**0.120**	**Inf**	**2.984**	**0.003**
Rice:Trinidad – Mixture:Trinidad	**-0.878**	**0.091**	**Inf**	**-9.622**	**<0.0001**
Maize:Trinidad – Mixture:Trinidad	**-1.235**	**0.104**	**Inf**	**-11.855**	**<0.0001**
Within grain, compare strain					
Wheat					
Wheat:TAQ – Wheat:Africa	**0.219**	**0.056**	**Inf**	**3.925**	**<0.0001**
Wheat:TAQ – Wheat:Queensland	0.085	0.054	Inf	1.584	0.113
Wheat:TAQ – Wheat:Trinidad	**0.124**	**0.055**	**Inf**	**2.248**	**0.025**
Wheat:Africa – Wheat:Queensland	**-0.134**	**0.057**	**Inf**	**-2.351**	**0.019**
Wheat:Africa – Wheat:Trinidad	-0.095	0.058	Inf	-1.629	0.103
Wheat:Queensland – Wheat:Trinidad	0.039	0.056	Inf	0.690	0.490

Variable	β	SE	DF	Z-ratio	*P*-value
Rice					
Rice:TAQ – Rice:Africa	0.048	0.098	Inf	0.489	0.625
Rice:TAQ – Rice:Queensland	-0.003	0.098	Inf	-0.035	0.972
Rice:TAQ – Rice:Trinidad	0.150	0.103	Inf	1.456	0.145
Rice:Africa – Rice:Queensland	-0.051	0.099	Inf	-0.518	0.604
Rice:Africa – Rice:Trinidad	0.102	0.104	Inf	0.981	0.326
Rice:Queensland – Rice:Trinidad	0.154	0.104	Inf	1.474	0.141
Maize					
Maize:TAQ – Maize:Africa	0.039	0.114	Inf	0.342	0.732
Maize:TAQ – Maize:Queensland	-0.120	0.110	Inf	-1.094	0.274
Maize:TAQ – Maize:Trinidad	0.197	0.122	Inf	1.617	0.106
Maize:Africa – Maize:Queensland	-0.159	0.111	Inf	-1.434	0.152
Maize:Africa – Maize:Trinidad	0.158	0.123	Inf	1.287	0.198
Maize:Queensland – Maize:Trinidad	**0.317**	**0.119**	**Inf**	**2.667**	**0.008**
Mixture					
Mixture:TAQ – Mixture:Africa	0.033	0.074	Inf	0.445	0.656
Mixture:TAQ – Mixture:Queensland	-0.019	0.073	Inf	-0.257	0.798
Mixture:TAQ – Mixture:Trinidad	-0.183	0.072	Inf	-2.533	0.011
Mixture:Africa – Mixture:Queensland	-0.052	0.074	Inf	-0.702	0.483

Variable	β	SE	DF	Z-ratio	*P*-value
Mixture:Africa – Mixture:Trinidad	**-0.216**	**0.073**	**Inf**	**-2.965**	**0.003**
Mixture:Queensland – Mixture:Trinidad	**-0.164**	**0.072**	**Inf**	**-2.284**	**0.022**

Table 2A.7 Differences of least squares means and confidence intervals for models predicting *S. oryzae* population responses of body weight variation as a function of phenotypic variation (PV), *S. oryzae* strain (hybrid strain TAQ, Africa, Queensland and Trinidad), the grain type (wheat, rice, maize and mixture of all three grains), sex, development period, offspring body weight. We report best parameter estimates (β), standard error (SE), degrees of freedom (DF), t- value, their 95% confidence interval (CI), and P-value. The colon separating variable names indicates interaction terms. Significant P-values are highlighted in bold.

Variable	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Phenotypic variation							
NV – PV	-0.004	0.005	273.087	-0.878	-0.014	0.005	0.381
Strain							
TAQ – Africa	0.000	0.007	273.640	0.028	-0.014	0.015	0.978
TAQ – Queensland	-0.003	0.007	273.174	-0.425	-0.017	0.011	0.671
TAQ – Trinidad	**0.017**	**0.007**	**265.452**	**2.250**	**0.002**	**0.031**	**0.025**
Africa – Queensland	-0.003	0.007	273.386	-0.453	-0.017	0.011	0.651
Africa – Trinidad	**0.017**	**0.007**	**253.490**	**2.351**	**0.003**	**0.030**	**0.019**
Queensland – Trinidad	**0.020**	**0.007**	**267.010**	**2.763**	**0.006**	**0.034**	**0.006**
Grain							
Wheat – Rice	**-0.057**	**0.014**	**194.027**	**-3.989**	**-0.086**	**-0.029**	**<0.0001**
Wheat – Maize	**-0.038**	**0.016**	**201.807**	**-2.383**	**-0.069**	**-0.007**	**0.018**
Wheat – Mixture	**-0.047**	**0.010**	**189.047**	**-4.800**	**-0.066**	**-0.028**	**<0.0001**

Variable	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Rice – Maize	**0.019**	**0.007**	**273.693**	**2.712**	**0.005**	**0.034**	**0.007**
Rice – Mixture	0.010	0.009	272.483	1.109	-0.008	0.029	0.269
Maize – Mixture	-0.009	0.011	270.274	-0.835	-0.030	0.012	0.405

Table 2A.8 Differences of least squares means and confidence intervals for models predicting *S. oryzae* population responses of total biomass as a function of phenotypic variation (PV), *S. oryzae* strain (hybrid strain TAQ, Africa, Queensland and Trinidad), the grain type (wheat, rice, maize and mixture of all three grains), sex, development period, offspring body weight. We report best parameter estimates (β), standard error (SE), degrees of freedom (DF), t- value, their 95% confidence interval (CI), and P-value. The colon separating variable names indicates interaction terms. Significant P-values are highlighted in bold.

Variable	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Phenotypic variation							
NV - PV	-0.557	0.713	267.006	-0.782	-1.961	0.846	0.435
Strain							
TAQ - Africa	1.728	1.047	267.100	1.650	-0.334	3.789	0.100
TAQ - Queensland	-0.777	1.025	267.028	-0.758	-2.795	1.241	0.449
TAQ - Trinidad	1.738	1.097	267.780	1.584	-0.422	3.897	0.114
Africa - Queensland	**-2.505**	**1.016**	**267.026**	**-2.465**	**-4.505**	**-0.504**	**0.014**
Africa - Trinidad	0.010	1.026	267.550	0.010	-2.010	2.030	0.992
Queensland - Trinidad	**2.515**	**1.052**	**267.667**	**2.391**	**0.444**	**4.586**	**0.018**
Grain							
Wheat - Rice	**28.055**	**1.226**	**267.372**	**22.889**	**25.642**	**30.468**	**<0.0001**
Wheat - Maize	**27.085**	**1.339**	**267.512**	**20.231**	**24.449**	**29.721**	**<0.0001**
Wheat - Mixture	**16.680**	**1.003**	**267.011**	**16.624**	**14.704**	**18.655**	**<0.0001**

Variable	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Rice - Maize	-0.970	1.025	267.049	-0.946	-2.988	1.049	0.345
Rice - Mixture	**-11.376**	**1.190**	**267.303**	**-9.560**	**-13.719**	**-9.033**	**<0.0001**
Maize - Mixture	**-10.406**	**1.296**	**267.448**	**-8.029**	**-12.958**	**-7.854**	**<0.0001**
Within strain, compare grain							
TAQ							
Wheat:TAQ - Rice:TAQ	**32.907**	**2.120**	**267.109**	**15.525**	**28.734**	**37.081**	**<0.0001**
Wheat:TAQ - Maize:TAQ	**32.339**	**2.163**	**267.170**	**14.950**	**28.080**	**36.598**	**<0.0001**
Wheat:TAQ - Mixture:TAQ	**23.027**	**2.010**	**267.002**	**11.455**	**19.069**	**26.985**	**<0.0001**
Rice:TAQ - Maize:TAQ	-0.568	2.022	267.011	-0.281	-4.549	3.412	0.779
Rice:TAQ - Mixture:TAQ	**-9.880**	**2.099**	**267.096**	**-4.707**	**-14.013**	**-5.748**	**<0.0001**
Maize:TAQ - Mixture:TAQ	**-9.312**	**2.145**	**267.155**	**-4.342**	**-13.535**	**-5.090**	**<0.0001**
Africa							
Wheat:Africa - Rice:Africa	**25.428**	**2.105**	**267.113**	**12.079**	**21.283**	**29.573**	**<0.0001**
Wheat:Africa - Maize:Africa	**24.476**	**2.157**	**267.162**	**11.349**	**20.230**	**28.723**	**<0.0001**
Wheat:Africa - Mixture:Africa	**14.539**	**2.017**	**267.012**	**7.208**	**10.567**	**18.510**	**<0.0001**
Rice:Africa - Maize:Africa	-0.952	2.015	267.008	-0.472	-4.919	3.015	0.637
Rice:Africa - Mixture:Africa	**-10.890**	**2.059**	**267.059**	**-5.290**	**-14.943**	**-6.837**	**<0.0001**
Maize:Africa - Mixture:Africa	**-9.938**	**2.099**	**267.100**	**-4.734**	**-14.071**	**-5.805**	**<0.0001**

Variable	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Queensland							
Wheat:Queensland - Rice:Queensland	**30.450**	**2.180**	**267.117**	**13.966**	**26.157**	**34.742**	**<0.0001**
Wheat:Queensland - Maize:Queensland	**28.415**	**2.231**	**267.212**	**12.739**	**24.023**	**32.807**	**<0.0001**
Wheat:Queensland - Mixture:Queensland	**18.698**	**2.009**	**267.001**	**9.309**	**14.743**	**22.652**	**<0.0001**
Rice:Queensland - Maize:Queensland	-2.035	2.048	267.022	-0.994	-6.066	1.997	0.321
Rice:Queensland - Mixture:Queensland	**-11.752**	**2.190**	**267.126**	**-5.367**	**-16.063**	**-7.441**	**<0.0001**
Maize:Queensland - Mixture:Queensland	**-9.717**	**2.242**	**267.222**	**-4.335**	**-14.131**	**-5.303**	**<0.0001**
Trinidad							
Wheat:Trinidad - Rice:Trinidad	**23.436**	**2.116**	**267.180**	**11.074**	**19.269**	**27.602**	**<0.0001**
Wheat:Trinidad - Maize:Trinidad	**23.111**	**2.225**	**267.261**	**10.387**	**18.730**	**27.491**	**<0.0001**
Wheat:Trinidad - Mixture:Trinidad	**10.455**	**1.983**	**267.008**	**5.271**	**6.550**	**14.360**	**<0.0001**
Rice:Trinidad - Maize:Trinidad	-0.325	2.030	267.017	-0.160	-4.322	3.672	0.873
Rice:Trinidad - Mixture:Trinidad	**-12.981**	**2.118**	**267.121**	**-6.130**	**-17.150**	**-8.811**	**<0.0001**
Maize:Trinidad - Mixture:Trinidad	**-12.656**	**2.218**	**267.196**	**-5.706**	**-17.023**	**-8.289**	**<0.0001**

Variable	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Within grain, compare strain							
Wheat							
Wheat:TAQ - Wheat:Africa	**7.685**	**2.029**	**267.022**	**3.788**	**3.691**	**11.679**	**<0.0001**
Wheat:TAQ - Wheat:Queensland	1.901	2.013	267.006	0.944	-2.063	5.865	0.346
Wheat:TAQ - Wheat:Trinidad	**9.556**	**2.019**	**267.208**	**4.733**	**5.581**	**13.530**	**<0.0001**
Wheat:Africa - Wheat:Queensland	**-5.784**	**2.014**	**267.007**	**-2.873**	**-9.749**	**-1.820**	**0.004**
Wheat:Africa - Wheat:Trinidad	1.870	1.995	267.135	0.938	-2.058	5.799	0.349
Wheat:Queensland - Wheat:Trinidad	**7.655**	**2.002**	**267.169**	**3.823**	**3.712**	**11.597**	**<0.0001**
Rice							
Rice:TAQ - Rice:Africa	0.206	2.028	267.024	0.102	-3.787	4.199	0.919
Rice:TAQ - Rice:Queensland	-0.557	2.053	267.010	-0.271	-4.599	3.485	0.786
Rice:TAQ - Rice:Trinidad	0.084	2.067	267.254	0.041	-3.985	4.153	0.968
Rice:Africa - Rice:Queensland	-0.763	2.041	267.004	-0.374	-4.781	3.255	0.709
Rice:Africa - Rice:Trinidad	-0.122	2.025	267.169	-0.060	-4.110	3.865	0.952
Rice:Queensland - Rice:Trinidad	0.641	2.062	267.205	0.311	-3.418	4.700	0.756
Maize							
Maize:TAQ - Maize:Africa	-0.178	2.027	267.019	-0.088	-4.170	3.814	0.930
Maize:TAQ - Maize:Queensland	-2.023	2.040	267.020	-0.992	-6.039	1.992	0.322

Variable	β	SE	DF	*t*- value	Lower 95% CI	Upper 95% CI	*P*-value
Maize:TAQ - Maize:Trinidad	0.327	2.090	267.283	0.156	-3.789	4.443	0.876
Maize:Africa - Maize:Queensland	-1.846	2.012	267.001	-0.917	-5.806	2.115	0.360
Maize:Africa - Maize:Trinidad	0.505	2.036	267.197	0.248	-3.504	4.514	0.804
Maize:Queensland - Maize:Trinidad	2.351	2.032	267.189	1.157	-1.650	6.351	0.248
Mixture							
Mixture:TAQ - Mixture:Africa	-0.803	2.048	267.050	-0.392	-4.836	3.230	0.695
Mixture:TAQ - Mixture:Queensland	-2.429	2.009	267.002	-1.209	-6.384	1.527	0.228
Mixture:TAQ - Mixture:Trinidad	-3.016	2.050	267.228	-1.472	-7.052	1.019	0.142
Mixture:Africa - Mixture:Queensland	-1.625	2.039	267.038	-0.797	-5.639	2.388	0.426
Mixture:Africa - Mixture:Trinidad	-2.213	2.020	267.119	-1.096	-6.190	1.763	0.274
Mixture:Queensland - Mixture:Trinidad	-0.588	2.041	267.210	-0.288	-4.606	3.430	0.774

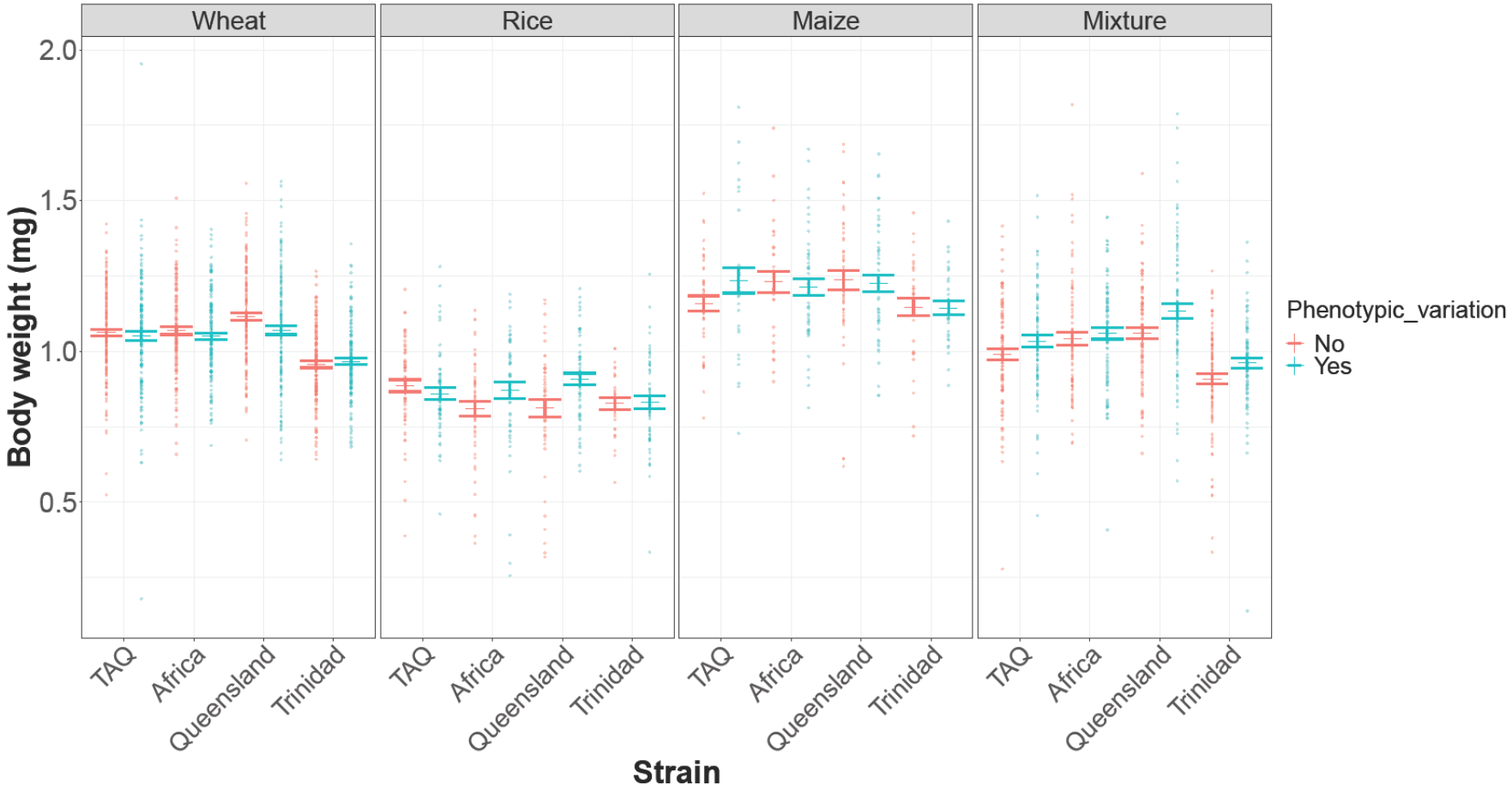

Figure 2A.1 The body weight of males for four *S. oryzae* (TAQ, Africa, Queensland and Trinidad) on four different grain types; (i) the grain which they were reared in: wheat (ii) novel grains: rice and maize and (iii) a novel and heterogeneous grain: mixed grain. Bars represent the mean ± standard error, whilst data points show the observed values of offspring body weight. Blue bars and points represent predicted body weight phenotypically variable size treatments (V) and red bars and points represent non-phenotypically variable size treatments (NV) of each.

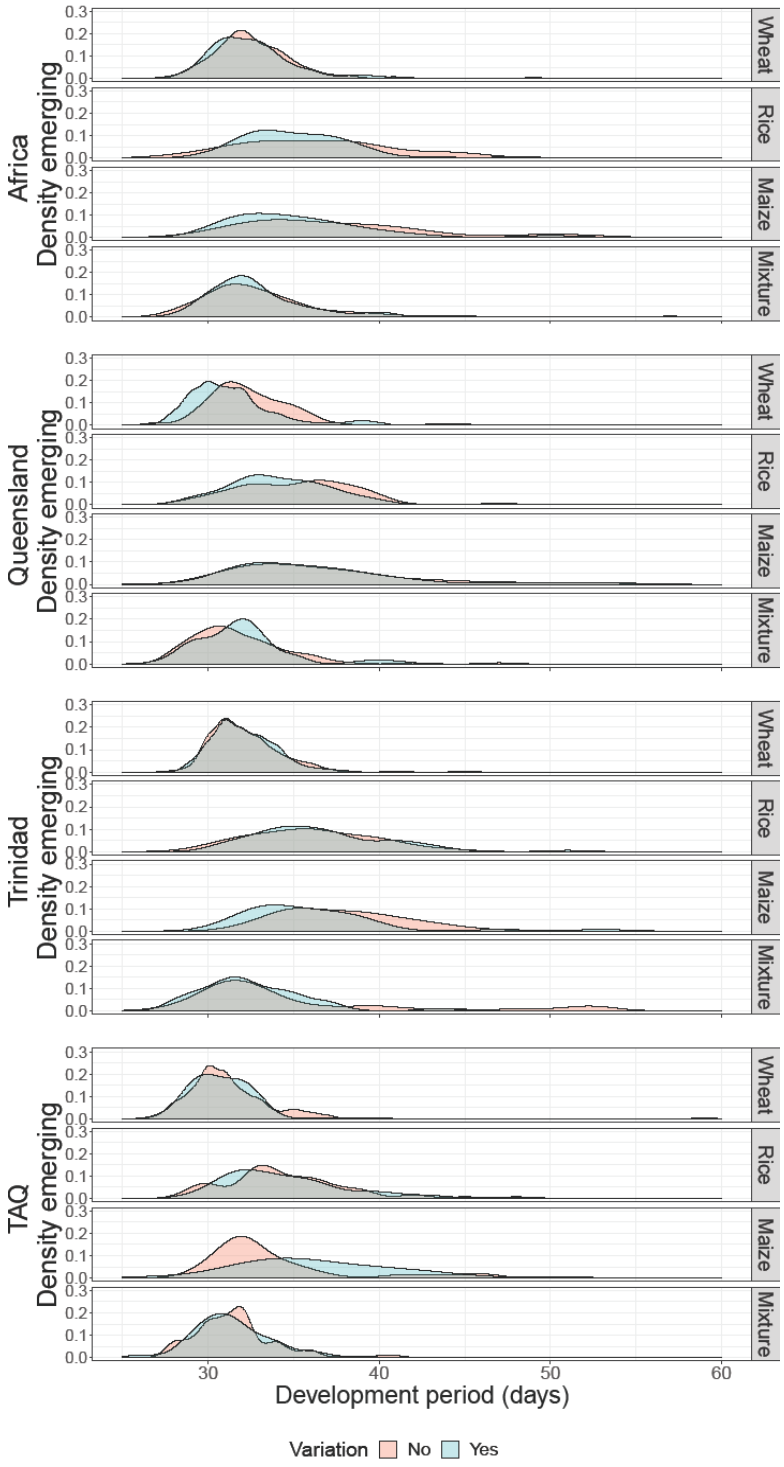

Figure 2A.2 The development period of males for *S. oryzae* (TAQ, Africa, Queensland and Trinidad) on four different grain types; (i) the grain which they were reared in: wheat (ii) novel grains: rice and maize and (iii) a novel and heterogeneous grain: mixed grain. Curves represent the number of individuals emerging on a given day. Blue curves represent predicted body weight phenotypically variable size treatments (V) and red bars and points represent non-phenotypically variable size treatments (NV) of each.

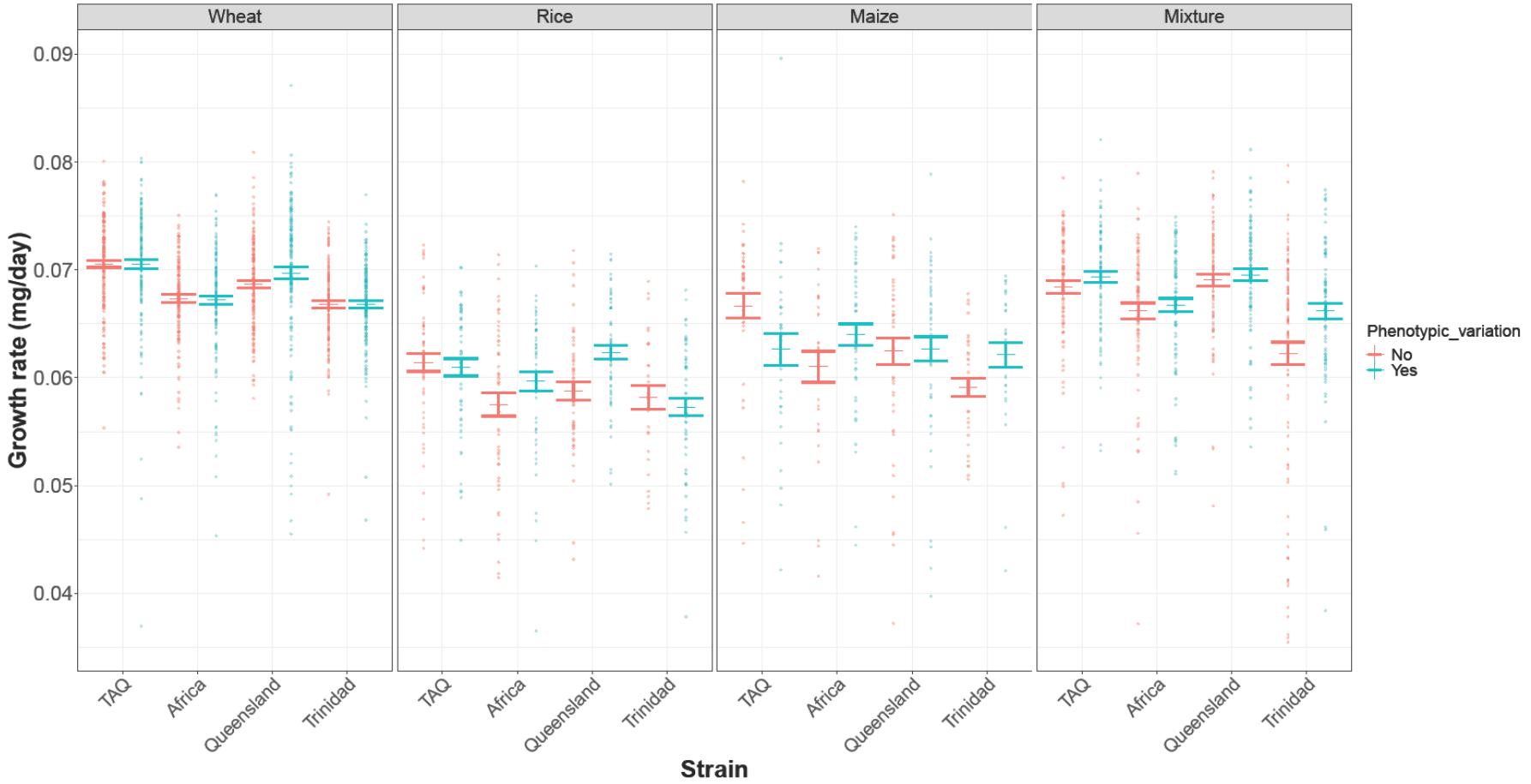

Figure 2A.3 The growth rate of males for four *S. oryzae* (TAQ, Africa, Queensland and Trinidad) on four different grain types; (i) the grain which they were reared in: wheat (ii) novel grains: rice and maize and (iii) a novel and heterogeneous grain: mixed grain. Bars represent the mean ± standard error, whilst data points show the observed values of offspring body weight. Blue bars and points represent predicted body weight phenotypically variable size treatments (V) and red bars and points represent non-phenotypically variable size treatments (NV) of each.

CHAPTER 3. Effect of humidity and temperature on the performance of three strains of *Aphalara itadori*, a biocontrol agent for Japanese Knotweed

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Running title: *Aphalara itadori* as a biocontrol for Japanese Knotweed

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Highlights

- Three strains of *Aphalara itadori* were tested under two environmental conditions
- More stressful environmental conditions slowed down psyllid development
- Biocontrol effectiveness was similar among strains, with no clear hybrid advantage

CHAPTER 3. Effect of humidity and temperature on the performance of three strains of *Aphalara itadori*, a biocontrol agent for Japanese Knotweed

Abstract

Japanese knotweed (*Fallopia japonica*) is a highly damaging invasive species affecting UK infrastructure and biodiversity. Under laboratory conditions, the psyllid *Aphalara itadori* has demonstrated its potential to be a successful biocontrol agent for *F. japonica.* However, this potential has not materialised in the field where long-term establishment of *A. itadori* has been unsuccessful and faces the added challenge of climate change. Intraspecific variation (variation among individuals of a species) has been shown to support establishment in alien species and improve resilience to changing environmental conditions, here we propose it could improve the performance of biocontrols. To test this possibility we compared the performance and impact on *F. japonica* of three strains of *A. itadori* with different genetic backgrounds, including a newly created hybrid. We hypothesize that genetic variability would be increased in hybrids resulting in greater biocontrol effectiveness (greater impact on plant growth). We also explored the potential influence of changing climate in performance, testing all strains under two humidity conditions (with the same temperature). Contrary to our expectation, the hybrid strain had the worst performance (slowest development rate and lower survival from egg to adult emergence) under both environmental conditions. Exposure to different strains of *A. itadori* did not result in consistent differences in plant growth, suggesting similar biocontrol effectiveness among strains. Under the drier, more stressful, conditions plants exposed to *A. itadori* had fewer leaves and accumulated less above-ground biomass.

Overall, our results suggest that genetic variability may not be the key to improve *A. itadori* biocontrol effectiveness, but that predicted climate change, which anticipates drier and hotter summers in the UK, could reduce the growth potential of *F. japonica* when exposed to *A. itadori*.

Keywords: Biological Control; Climate change; *Fallopia japonica*; Intraspecific Variation; Invasive Species; Japanese Psyllid; Saturation Deficiency Index.

Abbreviations

LTLR: long-term laboratory-reared strain

STLR: short-term laboratory-reared

SDI: Saturation Deficiency Index

3.1. Introduction

Invasive species are a significant problem in the United Kingdom, where they are estimated to cost the economy approximately £1.7 billion per annum (Booy et al., 2008; Williams et al., 2010). Invasive species are both damaging to the UK's infrastructure and to the native biodiversity. One of the most problematic invasive weeds in the UK is Japanese knotweed (*Fallopia japonica* [Houttuyn] Ronse Decraene), a species native to Japan. The lack of fertile *F. japonica* males in Britain, as determined from Random Amplified Polymorphic DNA (RAPDs) analysis, suggests that all *F. japonica* in the UK is derived from a single clonal individual that has reproduced through vegetative propagation (Hollingsworth and Bailey, 2000). This low genetic diversity however, has not hindered its invasive ability. *Fallopia japonica* has become established in a wide-range of habitats, and grows asexually from small fragments of underground root networks – rhizomes, weighing less than a gram (Bashtanova et al., 2009; Hollingsworth

and Bailey, 2000). These features, as well as its rapid growth rate, make *F. japonica* highly invasive in the UK.

There have been varying attempts to eradicate or control *F. japonica*. Manual or chemical removal can work at a local scale; however, the costs and time requirements make these methods unfeasible as long-term or large-scale management solutions. Herbicide use in parks and riparian areas where the plant is most prevalent has become less acceptable (Forman and Kesseli, 2003). Biological control is often proposed as an effective alternative tactic for invasive species, such as *F. japonica*. Reuniting an introduced weed with its host-specific natural enemies from their country of origin has resulted in successful suppression of many invasive weeds worldwide (Clewley et al., 2012; Schwarzländer et al., 2018). In comparison to other control methods, biocontrol can be used everywhere and is generally cost effective and environmentally friendly (Wittenberg and Cock, 2001).

The use of biocontrol agents for *F. japonica* in the UK has been explored by the non-profit organisation CABI, UK, since 2003. Initially, candidate species were identified from the Kyushu Island of Japan, the region from where the UK invasive *F. japonica* clones are thought to have originated (Djeddour and Shaw, 2010). Out of the 186 candidate arthropod species considered, *Aphalara itadori* Shinji (Hemiptera: Aphalaridae), otherwise known as Japanese knotweed psyllid, was found to be the best agent, since laboratory studies showed it to be host- specific (i.e. not affecting native plants) and highly damaging to *F. japonica*. However, despite its effectiveness under laboratory conditions (Grevstad et al., 2013), the establishment of viable populations in the field has been largely unsuccessful. A possible explanation for why field releases have failed is a lack of genetic and phenotypic variability in the batches of *A. itadori* that were released. Genetic bottlenecking is commonly implicated in the establishment failure of biocontrol agents (see review by Fauvergue et al., 2012). It is not unusual in biocontrol

programs for host-range testing for specificity and safety to require a long period of laboratory rearing. Indeed, in the UK, *A. itadori* was maintained in the laboratory from 2004 until its approval for release in 2010 (Shaw et al., 2009). Because the released *A. itadori* came from populations maintained under Japanese summer conditions at 22°C 13:11 hours day:night 50-85% humidity for at least six years (~66 generations), they may have become conditioned to the controlled environment room, as well as have potentially lost genetic diversity. This 'colony effect' of laboratory reared animals has been seen in other insect species, such as in *Drosophila* when undergoing laboratory selection experiments (Harshman and Hoffmann, 2000) and when comparing wild to laboratory cultures of *Drosophila* (Sgrò and Partridge, 2000), and also in *Anopheles gambiae* (Huho et al., 2007). As a result, the long-term laboratory-reared *A. itadori* could have been ill-prepared for dealing with the variability in the natural environmental conditions in the UK.

Intraspecific variation — the diversity of characteristics amongst individuals of a species (Cianciaruso et al., 2009) — can be an important factor aiding in the establishment of alien species (Forsman, 2014), but as mentioned above variability may be reduced in laboratory-reared organisms. Plant and animal species with higher levels of intraspecific genetic and phenotypic variation are more likely to establish successfully in new environments under laboratory, semi-natural and natural conditions, with the largest effects seen in natural experiments (Forsman, 2014). In addition, intraspecific variability can provide resilience to changes in climatic conditions (Reusch et al., 2005; Sgrò and Hoffmann, 2004). Under climate change, more variable populations are predicted to have an increased chance of containing individuals with genotypes that allow population persistence (Oliver et al., 2015) whereas locally adapted, less diverse populations are vulnerable because they have evolved traits to suite only local stress factors (Benito Garzón et al., 2011).

The establishment of *A. itadori* may also have been affected by the interaction of different climatic conditions. Hodkinson (2009) and pilot field experiments (CABI, unpublished data) have shown that *A. itadori* population dynamics, and therefore their potential for establishment in the UK, can be affected by expected rising temperatures and declining relative humidity. In the UK, under climate change, conditions are likely to become more stressful due to a predicted increase in temperature and decrease in humidity in the spring and summer (Murphy et al., 2010) when *A. itadori* are most active after hibernation (Hodkinson, 2009). Therefore, effective biocontrol requires consideration of how different environmental conditions could affect effectiveness and resilience to future changes in climate.

For this study we compared the performance of the strain used in historic biocontrol releases to two other strains with different genetic backgrounds. The first genetically different strain we tested was from the same locality as original strain (Kyushu in South Japan) but had a shorter laboratory-rearing history (2 years compared to 13 years). Using a newly collected wild type strain would have been desirable but was not possible due to the timing and cost of a new collection and quarantine space. The second genetically different strain tested was a new hybrid strain created from two distinct provenances of *A. itadori*. To create the hybrid we combined males from Kyushu and females from Hokkaido (North Japan; Grevstad et al., 2013). The Kyushu and Hokkaido strains of *A. itadori* are genetically distinct and both strain, as well as the hybrid, can be distinguished using neutral molecular markers (Andersen et al., 2016). We tested a hybrid as a potential approach to increase genetic variability and vigor (Birchler et al., 2006; Szűcs et al., 2012). However, hybridization can also have negative effects which could reduce the potential of this new hybrid strain (Heinze et al., 2019; Peer and Taborsky, 2005). The performance and impact on *F. japonica* of the three strains was tested under two environmental conditions that reflected standard laboratory growing conditions and a drier environment reflective of climate change predictions.

3.2. Material and methods

3.2.1. Aphalara itadori strains

We used three *Aphalara itadori* strains. Two, the LTLR and STLR strains, were established using adults collected from Kyushu, Japan (taken in 2004 and 2015 respectively). The hybrid strain was created by mating LTLR strain males with females from a *A. itadori* line collected in 2007 in Hokkaido, Japan and reared since that date at the Agriculture and AgriFood Centre (AAFC) in Lethbridge, Canada. The crossing of lines was completed in December 2016 at AAFC-Lethbridge under 16L:8D laboratory conditions. Second generation adult hybrids (N~200) were shipped to the UK and reared in CABI under standard laboratory conditions (see below). We used fourth generation hybrids for oviposition during the experiment. All three strains were reared on knotweed in 100 x 90 x 100cm Perspex cages (average ± SD: 16.9◦C ± 3.8◦C, 47.2% ± 10.7% RH and 14L:10D) in CABI's Egham quarantine greenhouse facility.

3.2.2. Experimental design and conditions

We tested two environmental conditions that we then characterized using empirical estimates of Saturation Deficiency Index (SDI), a measure of climate severity (Samways, 1987). In its simplest form, SDI it is the difference between the saturation vapour pressure (SVP) at maximum temperature, and the actual vapour pressure of a volume of air at maximum temperature (Green and Catling, 1971; Samways, 1987). The value of SDI increases with rising temperature and/or decreasing relative humidity. For our experiment, treatments were created by changing humidity within experimental cages. Plants under high SDI conditions, reflective of climate change predictions (hotter and drier), had dry capillary matting for the base of the cage and a 40 x 50cm gauze covered

hole at the back of the cage to increase ventilation. Plants in low SDI conditions had wet capillary matting for the base of the cage, watered with 800ml tap water every week, reflecting the standard laboratory growing conditions. We calculated empirical SDI values for each treatment cage following Abtew and Melesse, (2013) and Samways (1987):

$$SDI = \text{SVP}\left(\frac{100 - RH}{100}\right)$$ (Equation 1)

where RH is relative humidity, and SVP is saturation vapour pressure calculated based on temperature (T) as below:

$$SVP = 0.611\, e^{\left(\frac{17.27 \times T}{\text{T} + 237.7}\right)}$$ (Equation 2)

Humidity and temperature were recorded during the experiment at 30-minute intervals using LogTag Haxo-8 dataloggers placed inside the sleeve of one randomly selected plant per cage. We estimated SDI using the humidity and temperature recorded at each 30-minute interval. For each day we then identified the three highest SDI values and calculated the arithmetic mean per cage of those maxima over the duration of the experiment. This resulted in six SDI values (one per cage). We averaged the three highest values instead of using the single highest value to control for potential outliers. There are alternative methods of calculating SDI (see Green and Catling, 1971), but we found results were equivalent with all methods (Table 3A.1, Figure 3A.1).

Fifty-five days prior to the start of the first experimental batch, the rhizomes of 71 young *F. japonica* of uniform genetic stock (collected from a single *F. japonica* patch with vegetative reproduction) were cleaned and wet rhizome weights for each plant were obtained (average ± SD: 75.85g ± 36.06g). Each rhizome was potted in an individual plastic pot (14.7cm diameter) with a saucer (16.5cm diameter) and left to grow in a greenhouse under natural conditions (average ± SD: 21.0◦C ± 4.5◦C, 51.6% ± 12.4% RH and 14L:10D).

All experimentation was performed in quarantine glasshouses (average ± SD: 21.0◦C ± 4.5◦C, 51.6% ± 12.4% RH and 14L:10D). Due to space constraints in the glasshouses, the experiment was completed in three sequential batches over four months. For each batch, 14-15 days before the start of the experiment, 18 plants were cut to the fourth node above ground on the main stem and first node from the stem on branches, with additional stems cut to ground level. This allowed us to standardise above-ground measurements of biomass. Cut *F. japonica* material was collected and frozen, and dry weights later obtained for before and after above-ground weight comparisons. Plants were then randomly assigned a *A. itadori* strain, and six plants from each strain were placed into designated chambers for up to 8 days with 150 *A. itadori* adults to allow oviposition (n ≈ 25 *A. itadori* per plant).

After the oviposition period, the total number of eggs per plant was counted by searching the top and bottom of all leaves and nodes using a hand lens. Plants with very high numbers of eggs were removed from egging chambers earlier to avoid high egg density variation across treatments (batch one: one STLR low SDI and one hybrid high SDI plant; batch two: one STLR low SDI plant). Egg counts are minimum estimates because total counts would have required damaging the plant, which would have prevented the experiment. We make the assumption here that the number of visible eggs is proportionally related to the total number of eggs. Plants were then randomly assigned to a low or high SDI treatment, resulting in three plant replicates per strain per treatment per batch (experiment total: n = 9 plant replicates per strain per treatment, total n = 54). We used 1m long insect sleeves supported by bamboo hoops for each plant to prevent *A. itadori* from moving between plants (Figure 3A.2). Each plant was placed in a 16.5cm diameter saucer and irrigated twice a week manually to ensure *F. japonica* survival irrespective of treatment. Total adult counts began 37 days after plants were placed in treatment cages. Emergent adults were counted and removed using a manual aspirator every 6-7 days for six weeks to allow all adults from the eggs laid prior to the experiment

to emerge. Although the nymphal stages cause the most damage to plants (Djeddour and Shaw, 2010), accurately counting nymphs without removal is complicated, therefore we used adult counts to infer survival to adult emergence. After all adults were counted, we obtained wet weights of above ground and below ground plant biomass. Above ground plant material was then frozen and dry weights were later obtained.

3.2.3. Response variables: A. itadori performance and plant growth

We used survival to adult emergence (henceforth referred to as '*A. itadori* survival') and development rates to assess *A. itadori* performance. *Aphalara itadori* survival was adjusted for initial egg density, and was calculated as $100 * \frac{Adults}{Eggs}$, where *Eggs* was the total number of eggs counted before moving the plants to the experimental treatments, and *Adults* was the total number of emerged adults counted over the entire experiment for each plant. *Aphalara itadori* development rate was evaluated by comparing the number of adults for each plant (expressed as percentage of the total), counted at 1, 2 and 3 weeks after the first adult survival in each cage. Counts after week 3 were not considered to avoid counting second generation offspring emerging. One STLR plant from the low SDI treatment was removed as it had extreme adult *A. itadori* numbers emerging compared to initial eggs counted.

Due to space limitations in the quarantine glasshouses, we could not assess how SDI treatments affected plants without *A. itadori*. We evaluated impacts of *A. itadori* on *F. japonica* by measuring differences in above and below ground biomass, number of leaves and stem height. There was considerable variation in these traits between plants, thus, in the variables rhizome weight, maximum height and leaf number, we did not compare absolute growth but instead calculated relative growth as $100 * \frac{(Final - Initial)}{Initial}$, where *Final* was the measurement taken at the end of the experiment and *Initial* was the

measurement before the start of the experiment. For the variable above-ground weight, the *Initial* was taken as zero (plants were potted as rhizomes, without above ground material), and the *Final* was calculated as the sum of the material that had been removed just prior to the experiment (to standardize plant size) and the remaining material at the end. Both were measured as dry weights. Plant material was wrapped in foil and placed into an oven at 70-90ºC for 48h or until dried. As it was not possible to dry rhizomes before the experiment without killing the plant, change in below ground biomass was calculated using wet weights. The number of leaves was counted at the start and the end of the experiment. Stem height was measured using a ruler from soil level to the tallest standing point on the plant.

3.2.4. Data analysis

We evaluated the effect of strain and SDI on *A. itadori* survival, development and the four measurements of *F. japonica* growth using linear mixed effect regression models fitted with function 'lmer' from package *lme4* (Bates et al., 2015) in R version 3.4.3 (R Core Team, 2017). Table A2 lists the fixed and random effects considered for each model. In summary, all models included as a random factor the batch number (one, two or three) and, for *A. itadori* survival and development, also observer ID (authors CF and CP, and Kate Constantine contributed to egg counting). All models included SDI and strain as fixed predictors. In addition, models assessing plant growth included as covariates: total number of adults to control for variation in insect densities, and rhizome weight to control for initial plant conditions (except when modelling rhizome weight). Models of *A. itadori* survival also included the total number of eggs as a covariate. To model *A. itadori* development we used a B-splines analysis based on count week to allow for non-linear changes in development. We tested models with additive effects only, as well as with interactions between strain and SDI treatment. In the case of

development, Week was also tested for interactions (Table 3A.2). Models with interactions were only considered to be supported if interaction terms were significant (p-value < 0.05). We evaluated model assumptions (normality and heteroscedasticity) plotting residuals from tested models. We used post-hoc tests based on R function 'difflsmeans' and 'lsmeansLT' from package *lmerTest* (Kuznetsova et al., 2017) to contrast among strains.

3.3. Results

3.3.1. Aphalara itadori performance

Aphalara itadori survival varied among strains ($F_{2, 36.17}$ = 12.49, P < 0.001, n = 18, 17 and 18 for LTLR, STLR and Hybrid strains respectively; Table 3.1, Figure 3.1A). In particular, survival from egg to adult emergence was significantly lower in hybrids (predicted mean [95% confidence intervals]: 26.00% [10.99 – 41.01]) compared to LTLR (57.72% [41.86 – 73.57]) and STLR (54.79% [34.49 – 75.08]) strains, but LTLR and STLR did not differ (P = 0.68). SDI did not significantly affect *A. itadori* survival ($F_{1, 46.86}$ = 1.66, P = 0.20), but survival was proportionally higher in plants with fewer eggs suggesting a density dependence effect ($F_{1, 46.82}$ = 7.98, P = 0.007).

The proportion of adults emerging generally decreased from the first to the third week, with earlier emergence time under low SDI (higher humidity, $F_{1, 153}$ = 28.34, P < 0.001; Table 3.1). The LTLR strain had the fastest development rates, with notable difference under high SDI, with the LTLR strain having peak emergence in the first week compared to both the STLR and the hybrid strain which displayed peak emergence during the second week (Table 3.1, Figure 3.1B). There was an interaction between STLR and SDI,

with the majority of STLR adults emerging sooner under lower SDI ($F_{2, 153}$ = 6.69, P < 0.001).

Out of the four plant growth variables tested, leaf number was the only response variable which was influenced by another predictor besides strain and SDI (other predictors: total eggs, number of adults, week of emergence and initial rhizome weight; see Table 3A.2 for when these predictors were included in our models), where higher rhizome weights at the start of the experiment were associated with more leaves ($F_{1, 47.01}$ = 9.29, P = 0.004; Table 3.2). None of the variables we tested explained change in rhizome weight (Table 3.2; Figure 3.1A).

Table 3.1 Coefficient estimates for the model predicting *Aphalara itadori* adult survival to adult emergence as a function of total number of *A. itadori* eggs, Saturation Deficiency Index value (SDI), and *A. itadori* strain (LTLR = Long-term laboratory reared; STLR = short-term laboratory reared and Hybrid strain); and adult development as a function of time (in weeks), Saturation Deficiency Index value (SDI), and *A. itadori* strain. We report best parameter estimates (β), their 95% confidence interval (CI), *P*-value, and the number of plants used in each analyses (*N*). The strain reference level (e.g. 'LTLR') is indicated in parentheses. The colon separating variable names indicates interaction terms. Significant variables are highlighted in bold.

Variable	β	Lower 95% CI	Upper 95% CI	*P*-value
Survival (*N* = 53)*				
Intercept (LTLR)	66.05	50.23	81.88	<0.001
SDI	0.54	-0.28	1.37	0.204
STLR	-2.93	-16.71	10.86	0.679
Hybrid	**-31.71**	**-45.67**	**-17.76**	**<0.001**
Total eggs	**-0.02**	**-0.03**	**-0.01**	**0.007**
Development (*N* = 54)				
Intercept (LTLR: Low)	78.05	69.42	86.69	<.001
STLR	**-20.91**	**-30.29**	**-11.54**	**<.001**
Hybrid	**-10.23**	**-19.67**	**-0.78**	**0.035**
High	**-32.64**	**-40.26**	**-25.02**	**<.001**
LTLR: Week 1-2	**-56.54**	**-67.23**	**-45.85**	**<.001**
LTLR: Week 2-3	**-77.62**	**-88.31**	**-66.93**	**<.001**
STLR: Week 1-2	**33.07**	**19.98**	**46.16**	**<.001**
STLR: Week 2-3	**29.67**	**16.58**	**42.76**	**<.001**
Hybrid: Week 1-2	**14.72**	**1.63**	**27.81**	**0.029**
Hybrid: Week 2-3	**15.96**	**2.86**	**29.05**	**0.018**
High: Week 1-2	**53.17**	**42.48**	**63.86**	**<.001**
High: Week 2-3	**44.75**	**34.06**	**55.43**	**<.001**
Total Eggs	0	-0.01	0.01	1

*One STLR plant had extreme adult *A. itadori* numbers emerging was removed from analysis

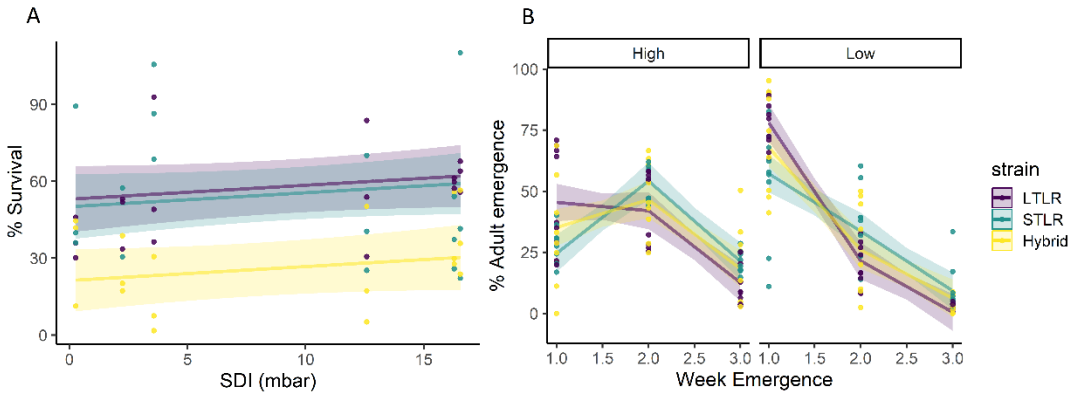

Figure 3.1 Relationship between *Aphalara itadori* performance in terms of (A) percentage *A. itadori* survival to adult emergence versus Saturation Deficiency Index (SDI) in treatment cages and (B) *A. itadori* development rate per week for high (more stressful) and low (less stressful) SDI treatments. Data points show the observed survival of three *A. itadorii* strains (LTLR = Long-term laboratory reared; STLR = short-term laboratory reared and Hybrid strain) grown on *Fallopia japonica*. Lines show the predicted relationship with SDI from (A) linear mixed effects model and (B) B-splines regression analysis based on count week. Shaded areas indicate 95% confidence intervals.

Table 3.2 Summary of models predicting percentage change in *Fallopia japonica* factors as a function of Saturation Deficiency Index (SDI), *Aphalara itadori* strain (LTLR = Long-term laboratory reared; STLR = short-term laboratory reared and Hybrid strain), the total number of *A. itadori* adults produced at the end of the experiment, and the initial rhizome weight. We report best parameter estimates (β), their 95% confidence interval (CI), *P*-value, and the number of plants used in each analyses (*N*). The strain reference level (e.g. 'LTLR') is indicated in parentheses. The colon separating variable names indicates interaction terms. Significant variables are highlighted in bold.

Variable	β	Lower 95% CI	Upper 95% CI	*P*-value
Rhizome weight (*N* = 54)				
Intercept (LTLR)	27.32	-3.89	58.53	0.109
SDI	0.22	-1.03	1.47	0.729
STLR	7.29	-13.86	28.44	0.503
Hybrid	12.77	-12.45	37.98	0.326
Number of Adults	-0.01	-0.06	0.04	0.770
Above Ground Weight (*N* = 46)*				
Intercept (LTLR)	57.97	27.22	88.73	0.020
SDI	**-0.87**	**-1.58**	**-0.17**	**0.020**
STLR	-2.01	-13.79	9.77	0.740
Hybrid	-4.00	-18.61	10.61	0.594
Number of Adults	-0.02	-0.05	0.00	0.106
Initial Rhizome Weight	-0.00	-0.13	0.12	0.940
Maximum Height (*N* = 54)				
Intercept (LTLR)	340.49	204.08	476.90	<0.001
SDI	-1.14	-8.30	6.01	0.755
STLR	**150.85**	**42.17**	**259.54**	**0.009**
Hybrid	**142.80**	**23.88**	**261.72**	**0.023**
SDI: STLR	**-16.27**	**-26.10**	**-6.45**	**0.002**
SDI: Hybrid	-7.67	-17.33	2.00	0.127
Number of Adults	-0.15	-0.32	0.02	0.081
Initial Rhizome Weight	-0.12	-0.94	0.69	0.769
Leaf Number (*N* = 54)				
Intercept (LTLR)	1622.59	493.61	2751.57	0.009

SDI	**-51.75**	**-93.79**	**-9.71**	**0.020**
STLR	-127.08	-833.21	579.04	0.726
Hybrid	-438.50	-1272.10	395.10	0.308
Number of Adults	-0.26	-1.88	1.36	0.754
Initial Rhizome Weight	**12.48**	**4.45**	**20.50**	**0.004**

*Eight *F. japonica* (three LTLR, one STLR and four Hybrid) had weights missing and were removed from analysis

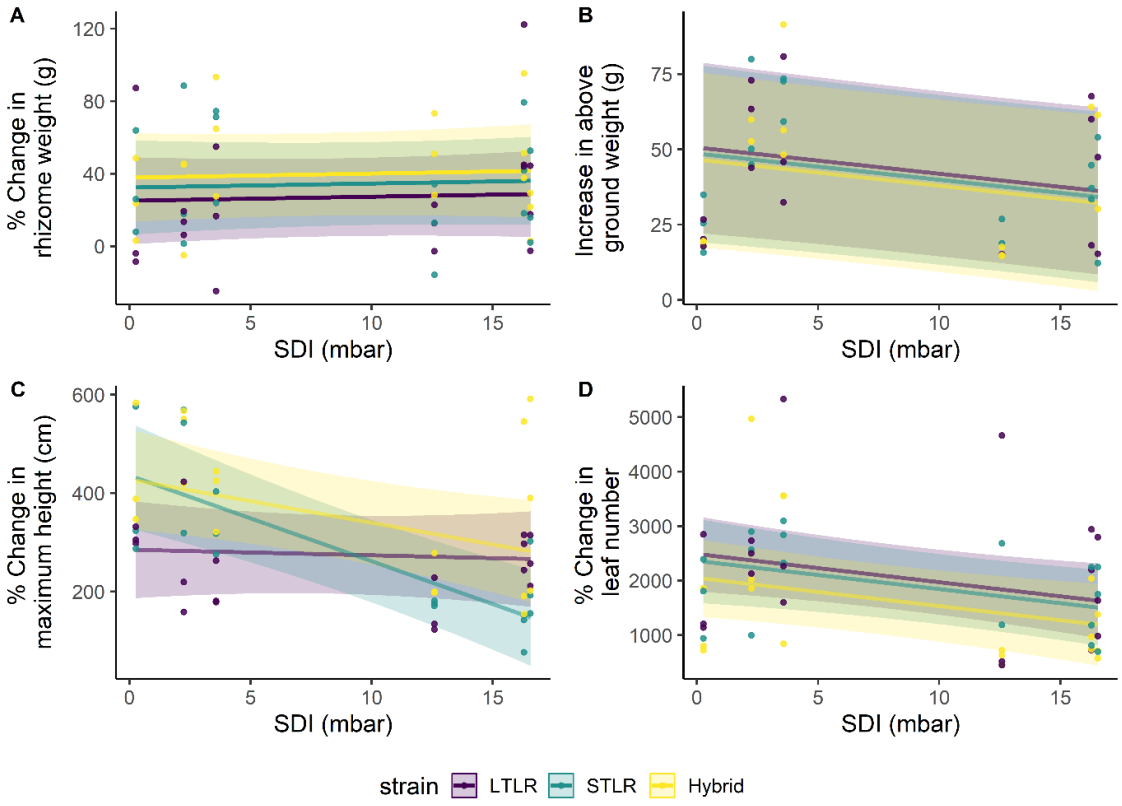

Figure 3.2 Relationship between growth of *F. japonica* versus Saturation Deficiency Index (SDI) in treatment cages. Data points show the observed survival to adult emergence of three *Aphalara itadori* strains (LTLR = Long-term laboratory reared; STLR = short-term laboratory reared and Hybrid strain) grown on *Fallopia japonica*. Plant growth was measured as either (A) rhizome weight, (B) above ground weight, (C) maximum height, and (D) leaf number. Lines show the predicted relationship with SDI from a linear mixed effects model with shaded areas showing 95% confidence intervals.

3.4. Discussion

Our study aimed to improve biocontrol of *F. japonica* by exploring the effectiveness of different *A. itadori* strains. We hypothesised that strains which had spent less time in the laboratory (STLR and hybrid strain) would have undergone less selection pressure to perform better under standard laboratory conditions, and therefore would perform better under altered climatic conditions. Previous studies have shown that laboratory rearing may lead to reduced genetic variability compared to wild stocks due to population bottlenecks and selection (Huho et al., 2007; Sgrò and Partridge, 2000), and therefore laboratory stocks tend to become more stress sensitive as selection for stress-related traits is relaxed (Hoffmann and Ross, 2018). Our results did not consistently support our predictions suggesting longer time in laboratory culture by itself is not affecting the performance of *A. itadori* biocontrol for *F. japonica*.

Among the strains, hybrids had lower survival and developed slower compared to the LTLR strain. Although the hybrid was created from two genetically different strains (Andersen et al., 2016), differences in the single-nucleotide polymorphisms (SNPs) may not have matched differences in functional gene regions linked to the traits we were assessing. In addition, although there have been studies which have shown improved hybrid fitness, for example in ornamental pear tree *Pyrus calleryana* (Culley and Hardiman, 2009), hybridisation in our study could have led to reduced, rather than improved, fitness. Between-population crosses from Bremgartewald and Spilwald strains of the black timber bark beetle, *Xylosandrus germanus,* were found to be less fit compared to inbred individuals (Peer and Taborsky, 2005). Hybrids from populations of the intertidal copepod species *Tigriopus californicus* also exhibited the negative effect of outbreeding depression, with hybrid fitness initially lower in terms of survivorship and morphology (Hwang et al., 2011). In our study, the hybrid strain was created from the

combination of males from the Kyushu strain, which performs best on *F. japonica* compared to other knotweeds, and females from the Hokkaido strain, which oviposit and develop well on *R. sachalinensis* (Grevstad et al., 2013). It is possible that hybrid breakdown may have occurred whereby the Hokkaido strain's adaptation and preference to living on *R. sachalinensis* was expressed in the hybrids, explaining the low survival to adult emergence observed in the hybrid strain compared to other strains. However, it is important to note that the hybrid was equal to the other two strains observed in terms of reducing the plant growth predictors assessed, and future work assessing more traits would further aid in determining the performance of hybrid strains.

Our study found that *Aphalara itadori* development was slower under high SDI, which has also been found for other psyllid species (see Hodkinson, 2009). Slower development could explain why plants exposed to *A. itadori* under stressful low humidity levels (high SDI) had lower growth in above-ground weight, height and number of leaves, compared to plants under high humidity levels. The more damaging nymphal stage of *A. itadori* is extended under slower development (Djeddour and Shaw, 2010) and therefore the per capita impact of individuals is likely to increase, potentially making them more effective biocontrol agents under high SDI conditions. Indeed, we found that the STLR strain developed slowest and had a greater impact on plant height under high SDI. However, this benefit could be offset by there being fewer generations per season, something that will need to be confirmed in future studies.

The findings that *A. itadori* survival was not influenced by SDI contrasts with other studies on other *A. itadori* species that have shown that high SDI leads to lower survival (Hall and Hentz, 2001; Hodkinson, 2009; McFarland and Hoy, 2001). These differences may reflect variation among species, but it is also possible that our drier conditions were not sufficiently stressful to induce mortality. The experiments were done within a greenhouse where conditions limited our ability to strictly control temperature and humidity.

Due to space limitations in the quarantine area we could not assess how environmental conditions affected plants without *A. itadori*. However, the reduced above-ground biomass and number of leaves observed in plants under high SDI could reflect more stressful conditions for the plants, especially as all plants were regularly watered, so only ambient humidity changed. If plants by themselves were not affected by the more stressful ambient conditions in the experiment, this suggests that *A. itadori* could be even more damaging when plant do suffer from high stress conditions in the field.

Notably, we found no effects of strain or SDI on rhizome weight. This could be because both insects and ambient humidity do not directly affect rhizomes, and nutrient availability in the soil was sufficient to avoid rhizome depletion associated to above ground growth. Since *F. japonica* is mainly spread by pieces of rhizome this highlights the challenge in developing an effective biological control to reduce the spread of this invasive plant.

Overall, our results do not support a beneficial role of intraspecific variation in the biocontrol effectiveness of *A. itadori*. Genetic work would be necessary to reveal if this is due to genetic variability being different from our expectation (lower in LTLR and highest in hybrids). Additional work under laboratory and field conditions would also be necessary to test a wider range of climate conditions (as responses are likely to be non-linear), to evaluate cross-generational changes including hybrid fitness after more generations, and to take into account additional factors such as predator avoidance and overwintering performance. Effectively controlling *F. japonica*, both above and below ground, is still the challenge ahead.

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Appendix

Table 3A.1 Alternative methods of calculating Saturation Deficiency Index value (SDI) adapted from (Green and Catling, 1971). Maximum temperatures were the maximum temperatures across the whole experiment.

	SDI Methods
1	Mean of 3 highest daily maximum temperatures with the mean of the three vapour pressures coinciding with the 3 highest maximum temperatures
2	Mean of the 3 SDI values (millibars) coinciding with the 3 highest maximum temperatures

Table 3A.2 All models tested to analyse the effects of strain and Saturation Deficiency Index value (SDI) on *Aphalara itadori* performance (survival to adult emergence and development) and *A. itadori* impact on *Fallopia japonica* growth (rhizome weight, above ground weight, maximum height and leaf number. * indicates tested interactions and models used are highlighted in bold.

Model	Fixed predictors	Random Factors
A.itadori Performance		
Survival		
S1	**Strain + SDI + total eggs**	**Batch + observer**
S2	Strain*SDI + total eggs	Batch + observer
Development		
D1	Strain + SDI + week emerge + total eggs	Batch + observer
D2	Strain*SDI + week emerge + total eggs	Batch + observer
D3	**Week emerge*(strain + SDI) + total eggs**	**Batch + observer**
D4	strain*(SDI + week emerge) + total eggs	Batch + observer
Impact on *F. japonica*		
Rhizome Weight		
R1	**Strain + SDI + total number of adults**	**Batch**
R2	Strain*SDI + total number of adults	Batch
Above Ground Weight		
A1	**Strain + SDI + total number of adults + before rhizome weight**	**Batch**
A2	Strain*SDI + total number of adults + before rhizome weight	Batch
Maximum Height		
H1	Strain + SDI + total number of adults + before rhizome weight	Batch
H2	**Strain*SDI + total number of adults + before rhizome weight**	**Batch**
Leaf Number		
L1	**Strain + SDI + total number of adults + before rhizome weight**	**Batch**
L2	Strain*SDI + total number of adults + before rhizome weight	Batch

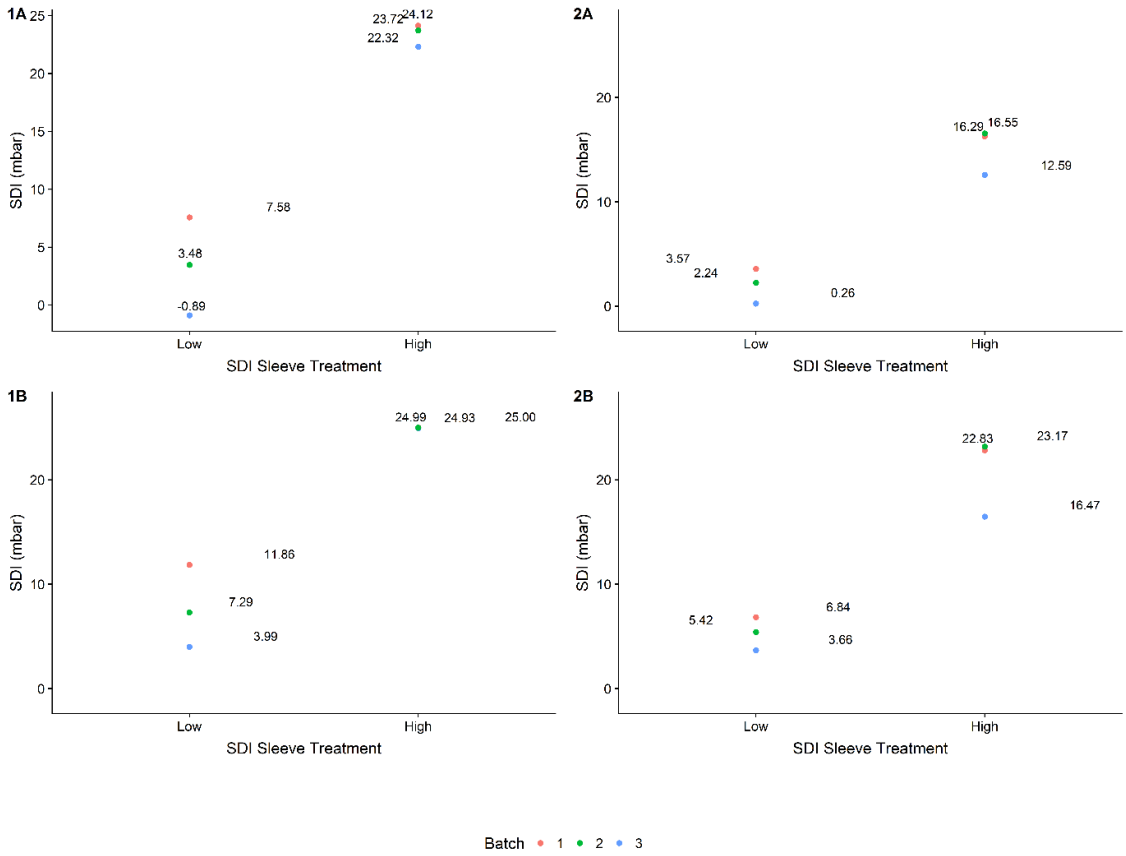

Figure 3A.1 Four methods chosen for calculating Saturation Deficiency Index value (SDI) adapted from (Green and Catling, 1971). Points are the calculated SDI values of dataloggers for each experimental batch. Each datalogger was placed in one sleeve within a treatment cage.

(1A) SDI was firstly calculated per day by taking the mean of the top three temperature values and its corresponding relative humidity values (RH). The final SDI value assigned to the batch was the average SDI for the whole experiment. (1B) SDI was firstly calculated per day by taking the mean of the top three temperature values and corresponding RH values. The final SDI value was than assigned by calculating the mean of the top three SDI values for the whole experiment. (2A) SDI values were calculated for each reading (30min) and the mean of the highest three SDI values was obtained. The final SDI value assigned to the batch was the average SDI for the whole experiment. (2B) SDI values were calculated for each reading (30min) and the mean of the highest three SDI values was obtained. The final SDI value assigned by calculating the mean of the top three SDI values for the whole experiment.

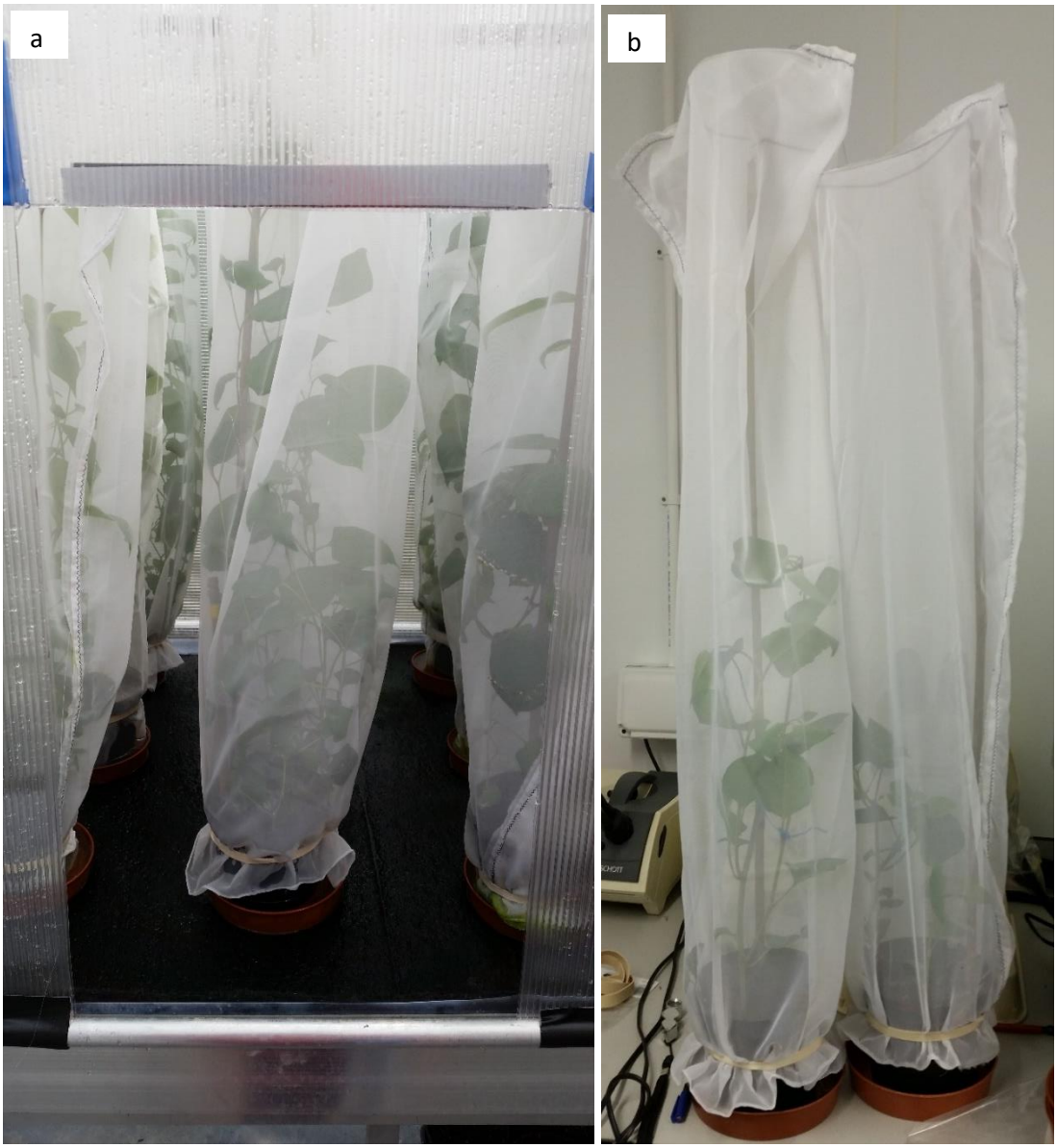

Figure 3A.2 Experimental *Fallopia japonica* plants. a) For the experiment, plants were placed in a 16.5cm diameter saucer within a humidity cage with capillary matting. They were irrigated twice a week manually; b) after egg counts, plants were covered in 1m long insect sleeves, tied with elastic bands and supported by bamboo halos to avoid *Aphalara itadori* escaping.

CHAPTER 4. Biocontrol agent efficacy is affected by intraspecific variation in pests: a study on the parasitoid wasp *Dinarmus basalis* and two morphs of its host the cowpea weevil *Callosobruchus maculatus*

Running title: Biocontrol agent efficacy is affected by intraspecific variation

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CHAPTER 4. Biocontrol agent efficacy is affected by intraspecific variation in pests: a study on the parasitoid wasp *Dinarmus basalis* and two morphs of its host the cowpea weevil *Callosobruchus maculatus*

Abstract

The cowpea weevil *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae) is a pest of several crops including cowpea *Vigna unguiculata* (L.) Walp (Fabaceae), an agriculturally important legume throughout the tropics. The solitary ectoparasitoid wasp *Dinarmus basalis* (Rondani) (Hymenoptera: Pteromalidae) has been proposed as an effective and low-cost biological control agent of *C. maculatus*. However, cowpea weevils naturally have two morphs: flight (active) and flightless (inactive), which have not been compared and could vary in their susceptibility to the wasp. The weevil morphs show differences in morphology and life-history with flight forms being generally dispersers with lower fecundity that tend to increase in frequency when population density and intraspecific competition are high. Here we explored whether the larvae from these two different weevil morph types had different effect on the behaviour, life-history or overall effectiveness of *D. basalis* as a biological control, and whether this, in turn, resulted in differential impact of the two *C. maculatus* morphs on stored cowpea. In a controlled laboratory experiment we tested the performance of *C. maculatus* offspring produced from flight vs flightless parent morphs with and without *D. basalis*. We found *D. basalis* was most effective at reducing larvae survival of the offspring of flight morph parents, but did not differentially affect the development period or the final adult

population size of weevil offspring from different parent morphs. The behaviour and life-history of *D. basalis* were also similar with each weevil morph. Despite having negative impacts on the weevils, the presence of *D. basalis* did not significantly reduce the amount of bean consumed by weevil larvae. In fact, the presence of *D. basalis* actually led to more bean damage (higher bean weight loss) by the offspring of flight morph parents. While future work will be necessary to investigate the long-term potential of *D. basalis* as a biocontrol, here we show the wasp affected offspring produced by both *C. maculatus* morphs, but while wasps caused lower survival of flight morph offspring (which over time could reduce population size), the impact in terms of bean loss was not be prevented in the short term. These complexities highlight the need to explicitly consider intraspecific variation when trialling potential biological controls for pest species, especially those displaying polymorphism.

Keywords: Intraspecific variation; Cowpea; Cowpea weevil; Parasitoid; Pest species; Bean pest; Pest management; Polymorphism; Phenotypic plasticity

4.1. Introduction

The cowpea weevil *Callosobruchus maculatus* (Fabricus) (Coleoptera: Chrysomelidae: Bruchinae) is a major pest of pulses worldwide, including the agriculturally important bean legume, cowpea *Vigna unguiculata* (L.) Walp (Fabaceae). Cowpea and other legumes are high in protein and affordable, and are an important part of the diet of many humans especially in tropical regions. *Vigna unguiculata* is particularly important because is drought tolerant (Mishili et al., 2009) which makes it a profitable plant in West African countries, including Ghana (Barde et al., 2014; Fujii et al., 1990; Kormawa et al., 2002). However, *V. unguiculata* is at risk of infestation by *C. maculatus* at every stage

of its production, from the field to storage, with the storage stage being the most affected (Tiroesele et al., 2015).

Cowpea weevil populations can exponentially increase in size and infect up to 90% of stored cowpea beans in just three to six months, reducing bean weight by 30-60% (Caswell, 1981; Van Alebeek and Wau, 1996). The beans left after heavy infestation are mostly inedible and not viable for replanting, which presents a serious loss for farmers (Messina et al., 2019). A variety of control methods have been applied including pesticides (Boeke et al., 2004; Kamanula et al., 2011), gamma radiation (Ibrahim et al., 2017), fumigation (Korletey, 2009; Ofuya et al., 2010), hermetic storage (Murdock et al., 2012), temperature regulation (Loganathan et al., 2011), ash (Kormawa et al., 2002; Wolfson et al., 1991) and regulating CO_2 in sealed nylon and surlyn storage bags (Fujii et al., 1990). For the past four decades chemical control methods has been preferred as they generally work and are easily accessible, despite the fact that their effectiveness can be greatly affected by the temperature, strain and even the bean on which *C. maculatus* develops (Gbaye and Holloway, 2011).

Alternative biological control agents have also been tested to stop or reduce the impact of cowpea infestations. For the pest weevil *C. maculatus*, the parasitoid wasp *Dinarmus basalis* Rond. (Hymenoptera: Pteromalidae) has been identified as a potentially effective biological control candidate (Ketoh et al., 2002), which could also control other storage pest such as the closely related weevil species *Callosobruchus chinensis* (L.) (Hossain et al., 2014). The wasp *D. basalis* is an ectoparasitoid and a common natural enemy of *C. maculatus* in field and in storage. The wasp oviposits its eggs on fourth instar larvae, the wasp offspring then feed on the weevil body fluids until they are ready to emerge as adults (Qumruzzaman and Islam, 2005; Sankara et al., 2014). Although this biological control is only effective after cowpea beans are infected with *C. maculatus* larvae, *D. basalis* has been found to be highly successful at decreasing weevil population size,

which could reduce bean damage. It is reported that the parasitoid is capable of suppressing up to 85% of the larvae of the cowpea weevil (Islam, 1998).

Understanding pest biology is vital for developing effective management strategies (Barde et al., 2014; Bawa et al., 2017). *Callosobruchus maculatus* naturally displays considerable intraspecific variation (Southgate et al., 1957). There are two distinct *C. maculatus* morphs: an active, long-winged flight form; and an inactive, short-winged flightless form (Southgate et al., 1957). The ratio of these morphs is influenced by larval density with the flight morph more likely to occur in crowded conditions, but also responding to environmental triggers such as temperature, moisture and photoperiod (Arnold et al., 2012; Sano, 1967; Utida, 1972). The morphs not only differ in their morphology but also show differences in life-history, behaviour, and physiology (Sano, 1967). Flightless morphs are more fecund (laying more eggs per female in a lifetime), have shorter life spans and reach sexual maturity earlier than their flight morph counterparts (Utida, 1972). The different traits between the two morphs are thought to be adaptations to the production process of cowpea beans, with the flight morph displaying dispersal traits most suitable to locate new sites in the field, whilst the flightless morph displays traits that are adapted to natural pressures that are selectively beneficial (e.g. maximize reproduction) for life within artificial stores (Bardner, 1982). The trait differences are thought to be determined by genetic and environmental factors (Bardner, 1982) therefore there is likely to be some inheritance of morph traits from parent to offspring.

The presence of flight polymorphs is common to other insects (Arnold et al., 2012), and Appleby and Credland (2001) noted that polymorphisms could affect how populations respond to any control methods. Polymorphisms, and more widely variation among individuals of the same species, represent intraspecific variation, which has been shown to influence population persistence (Kristensen et al., 2008; O'Grady et al., 2006; Vilas

et al., 2006), growth (Pelletier et al., 2007), and may even affect species interactions (Okuyama, 2008). Indeed, intraspecific variation in *C. maculatus* has been shown to influence their impact and response to resistant strains of cowpea (Appleby and Credland, 2003). Additionally, *C. maculatus* morphs have been shown to respond differently to infested beans, with the flight females significantly preferring infested to uninfested cowpea, whereas flightless females had no preference (Arnold et al., 2012).

Differences between *C. maculatus* morphs have potentially important consequences for the impact and management of this pest. For example, dispersal by the flight morph could result in wider spread of an infestation, particularly in rural areas where sealed storage facilities are rare and unaffordable, while higher fecundity in the flightless form could lead to higher, but localized, damage. Few studies (those cited above and Oyeniyi et al., 2015) had evaluated the distinct impact from each morph. To our knowledge, no previous work has tested whether morphs vary in their susceptibility to the proposed biological control, *D. basalis* and whether any differences in susceptibility and life-history between morphs can in turn, lead to different damage to stored cowpea beans. In the present study, we address these questions to determine if differences between populations of flight and flightless *C. maculatus* morphs affect biological control success of the larval parasitoid *D. basalis*. We compare the population size, survival, development, and impact (stored bean damage) of *C. maculatus* offspring from flight vs. flightless parents in the presence and absence of the wasp. We also compared wasp performance (behaviour, survival and population size) when parasitising offspring from flight vs. flightless parents. Since flight morphs have higher crude fat content and a higher biomass as adults (Chaudhuri, 2005; Utida, 1972), we hypothesised that the flight morph larvae would be favoured by the parasitoid wasp, and therefore be more affected by the presence of the biological control.

4.2. Material and Methods

4.2.1. Experimental design

We used a laboratory colony of the Cameroon strain of *C. maculatus*. This colony has been kept at the University of Reading for ~360 generations under control conditions (25 - 30°C temperature and 55 - 60% humidity). Since *C. maculatus* morphs can be density dependent, we used subpopulation culture jars with varying amounts of cowpea bean (15, 45, 75 and 105 g) to gather individuals of each morph, that were set up roughly 3.5 months prior to start of the experiment. To start the experiment, we selected beans with visible *C. maculatus* eggs from each culture jar. Each bean was then placed in a separate Eppendorf tube with a small breathing hole punctured at the top of the tube. These beans were checked several times a day for adult emergence, in order to obtain unmated individuals. Once an emerged adult was detected it was sexed, classified as either flight or flightless morph (see Table 4A.1 & Table 4A.2 for classification protocols), and then stored into separate morph and sex stocks. If multiple adults were found together in an Eppendorf, all were removed from the experiment to ensure only virgin adults were used.

Once enough virgin males and females were starting to emerge, we prepared two Petri dishes (one per morph), and placed 260 female and 60 male virgin *C. maculatus* adults in each. These adult had emerged three to eight days prior to allow flight form adults to reach sexual maturity (Utida,1972) and were then allowed to freely mate for 24 hours. We used an approximate 5:1 female:male sex ratio to reduce male-male competition. After 24 hours, we placed six presumably mated females of the same morph into an experimental Petri dish containing ten cowpea beans (total bean weight 16.4-16.9 g). Females were allowed to oviposit for 24 hours, after which they were removed. We created 20 replicate treatment Petri dishes per morph (120 females per morph), and also

set-up 15 control Petri dishes containing only bean to evaluate bean weight loss in the absence of the weevils.

Seventeen days after the weevil females had laid their eggs on the treatment Petri dishes (optimum larval stage for wasp oviposition; Kwasi Asante, unpublished data), we introduced two mated *D. basalis* females into 10 randomly selected treatment dishes, for each morph. Mated *D. basalis* females were randomly selected from a pool of ~100 females and ~100 males that had been allowed to mate for 48 hours. All *D. basalis* had been reared under control condition (25 - 30°C temperature and 55 - 60% humidity) at the University of Reading for five generations from wild-caught individuals collected from Ghana in June 2019. Mated female *D. basalis* remained in the treatment dishes for 24 hours. During the first 4.5 hours, every 30 minutes we noted for each Petri dish if females were on or off the beans, and if at least one female was on the bean, we classified their behaviour as drumming (otherwise referred to walk-antennating, where *D. basalis* was seen to palpate or “drum” the seed with its antennae in order to locate the host within the seed; Kumazaki et al., 2000; Mohamad et al., 2013), ovipositing (where the female was seen drilling through the seed with her ovipositor in order to make contact with the host and deposit her eggs; Kumazaki et al., 2000; Mohamad et al., 2013), or other.

After *D. basalis* females were removed, we separated and counted the number of *C. maculatus* eggs on each bean prior to placing each in an individually labelled Eppendorf with a small breathing hole punched at the top. Control beans were also placed in individual Eppendorf tubes to determine the average bean loss per Petri dish due to storage. We then checked daily for emerged *C. maculatus* and *D. basalis* in all wasp treatment beans, and in six of the 10 beans in each no-wasp replicate (time constraints preventing daily monitoring of all beans). All emerged weevil and wasp adults were removed from the tube, classified into a morph type (in the case of the weevils), sexed, and placed into a labelled Eppendorf with an air hole. All separated adults were

monitored daily to record time of death. For the no-wasp beans that were not monitored daily we counted, identified the morph, and sexed all emerged adults at the end of the experiment. After six days of no new adult emergence, we assumed that all insects had emerged from the bean and we weighed the bean from each treatment and control replicates. The final bean weight of one replicate was incorrectly noted and this replicate was removed from analysis of bean loss.

4.2.2. Data analysis

We measured the following response variables for each replicate: bean *water loss* (calculated as the average bean weight loss in control Petri dishes), *total bean loss* (the difference between initial and final bean in grams and includes the weight loss due to weevils feeding and the *water loss)*, *weevil population size* (the total number of emerged *C. maculatus* adults); and *per capita bean loss* (*total bean loss* divided by *weevil population size*). We also measured the following response variables per individual bean (Eppendorf) or per individual adult weevil: *weevil larval survival* (*Weevil population size* divided by number of *C. maculatus* eggs), *weevil development period* (time in days from start of the experiment to adult emergence), *weevil adult lifespan* (time in days from adult emergence to death). The *egg number* (total number of *C. maculatus* eggs laid in each Petri dish treatment replicate), *offspring morph* (the morph of each individual offspring which emerged), and *proportion flightless* (the proportion of offspring which were of the flightless morph per replicate) were also noted. For *D. basalis* performance we calculated the following response variables for each Petri dish treatment replicate: *wasp population size* (the total number of emerged adult *D. basalis*), *drumming behaviour* (the number of times drumming behaviour was observed) and *ovipositing behaviour* (the number of ovipositing behaviours was observed). Additionally, for wasp response variables per individual, we measured: *wasp development period* (time in days from

when wasps were introduced to Petri dish treatment replicated to adult emergence) and *wasp adult lifespan* (time in days from adult emergence to death).

We used regression models to determine which factors influenced each response variable. The parent morph was included as a predictor in all models, and the presence of *D. basalis* was a predictor in all *C. maculatus* response models. For all responses except *total bean loss, per capita bean loss* and *egg number,* models included the number of *C. maculatus* eggs (total per Petri dish or per Eppendorf depending on how the response was measured) as a control fixed predictor (Table 4A.4 & Table 4A.5). Other predictors included in individual models are described in Table 4A.4 & Table 4A.5. For each response we fitted two models: one with predictors as additive effects, and one testing the interaction between parent morph and presence of *D. basalis*. Models with interactions were considered to be supported if the interaction coefficient had a P-value < 0.05. To account for variability within Petri dishes and Eppendorfs, models for response variables measured at the Eppendorf or individual level included Petri dish name as a random effect. Additionally, models for responses measured at the individual level included Eppendorf name as a random effect.

We used linear mixed effect regression models for *development period* and *adult lifespan* of both *C. maculatus* and *D. basalis* and used generalized linear mixed effects regression models for *C. maculatus larvae survival*, *D. basalis drumming* and *ovipositing behaviour* and *offspring morph*. Models were fitted with the functions 'lmer' and 'glmer' (family binomial*)* from package *lme4* (Bates et al., 2015) in R version 3.4.3 (R Core Team, 2017). Variables measured at the Petri dish level did not include random factors, and were evaluated using generalised linear models fitting with the function 'lm' (*C. maculatus egg number, percentage flightless, bean loss* and *bean loss per capita)*, and 'glm' Poisson (*C. maculatus* and *D. basalis population size*) from *stats v3.6.2* package (R Core Team, 2020). We evaluated model assumptions (normality and

heteroscedasticity) and potential outliers plotting residuals from tested models. Post-hoc tests were performed to evaluate differences between flight and flightless parent morphs, and presence and absence of *D. basalis* using functions 'difflsmeans' and 'lsmeansLT' from package *lmerTest* (Kuznetsova et al., 2017) for 'lmer' models, and 'emmeans' from the package *emmeans* (Lenth, 2020) for 'glmer' and 'glm' models.

4.3. Results

4.3.1. Variation between offspring of weevil morphs

Parent morph had a significant effect on several weevil offspring responses (Figure 4.1), with flightless morph parents producing on average offspring with longer *development periods* and shorter *adult lifespan* (Figure 4.1C & D; Table 4.1 & Table 4.2). As described above, offspring belonging to different parent morphs also differed in their response to the presence of wasps, with reduced *larval survival* and higher bean damages for offspring of flight parents, when in the presence of wasps, but no differences when there were no wasps present (Figure 4.1B; Table 4.2). Parental morph did not influence offspring *population size* (Figure 4.1A; Table 4.2), but as expected, the number of offspring emerging as adults was larger in replicates with higher initial egg counts (Table 4.2). We also observed that flight parents oviposited more eggs (mean ± SD: 18.45 ± 5.03) than flightless parents (14.00 ± 4.44) within 24h (Figure 4A.1A; Table 4A.6). Offspring morph was partly influenced by parental morph, with flightless parents producing a higher percentage of flightless offspring, while flight parents were more likely to produce flight offspring (Figure 4A.1B & C; Table 4A.6 & Table 4A.7).

Table 4.1 Anova outputs for linear mixed effects regression models predicting cowpea weevil *C. maculatus* responses *development period* and *adult lifespan* as a function of the main predictors: parent morph (flightless or flight) and presence of *D. basalis* (yes or no). Some models also included as predictors: *C. maculatus* egg number and *C. maculatus* emergence morph. We report the sum of squares (sum sq), the arithmetic mean (mean sq), degrees of freedom in the numerator (numDF), degrees of freedom in the denominator (denDF), F-value and P-value for variables. Interaction terms are indicated by two variable names separated by a colon. Significant variables and interactions are highlighted in bold.

Variable	Sum sq	Mean sq	NumDF	DenDF	*F*-value	*P*-value
Development period						
Parent morph	**50.230**	**50.230**	**1**	**240.230**	**13.199**	**<0.0001**
Presence of wasp	**24.239**	**24.239**	**1**	**247.210**	**6.369**	**0.012**
Eppendorf no eggs	**27.592**	**27.592**	**1**	**250.060**	**7.250**	**0.008**
Adult lifespan						
Parent morph	**1122.150**	**1122.150**	**1**	**3310.300**	**49.252**	**<0.0001**
Presence of wasp	6.760	6.760	1	314.600	0.297	0.586
Eppendorf no eggs	**95.720**	**95.720**	**1**	**303.000**	**4.201**	**0.041**
Weevil emergence morph	**1002.380**	**1002.380**	**1**	**320.100**	**43.995**	**<0.0001**

Table 4.2 Anova outputs for generalized linear mixed effects regression models predicting the *bean loss* and *per capita bean loss*, *weevil population size* and *weevil larvae survival*, as a function of the main predictors: *C. maculatus* parent morph (flightless or flight) and presence of *D. basalis* (yes or no). Some models also included as predictors: total *C. maculatus* count per Petri dish replicate, the ratio of flightless offspring per Petri dish replicate and *C. maculatus* egg number per Petri dish replicate. We report Chi-square statistic (Chisq), degrees of freedom (DF) and P-value. Interaction terms are indicated by two variable names separated by a colon. Significant variables and interactions are highlighted in bold.

Variable	Chisq	Df	*P*-value
Bean loss			
Parent morph	**4.683**	**1**	**0.030**
Presence of wasp	1.820	1	0.177
Total Petri weevil count	2.574	1	0.109
Petri percent flightless	2.005	1	0.157
Parent morph:Presence of wasp	**5.475**	**1**	**0.019**
Per capita bean loss			
Parent morph	0.300	1	0.584
Presence of wasp	**3.878**	**1**	**0.049**
Petri percent flightless	0.483	1	0.487

Parent morph:Presence of wasp	3.075	1	0.080
Population size			
Parent morph	0.370	1	0.543
Presence of wasp	**14.206**	**1**	**<0.0001**
Petri no eggs	**41.327**	**1**	**<0.0001**
Larvae survival			
Parent morph	**7.005**	**1**	**0.008**
Presence of wasp	**9.701**	**1**	**0.002**
Parent morph:Presence of wasp	**6.623**	**1**	**0.010**

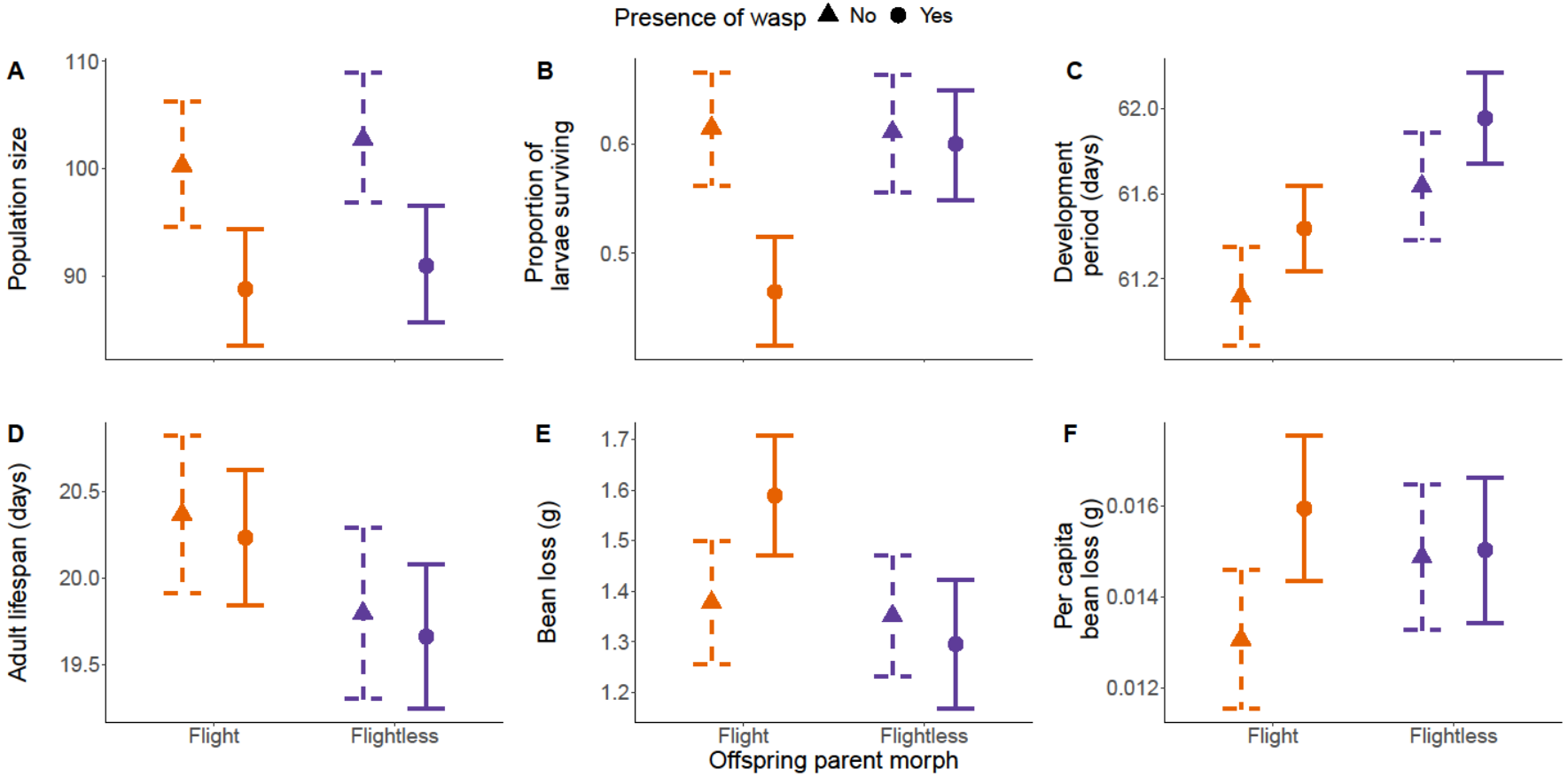

Figure 4.1 Cowpea weevil *C. maculatus* performance traits. We show the estimated model mean (symbol) and 95% confidence intervals (lines) for each treatment. Dashed lines with triangle symbols represent treatments without *D. basalis*, and solid CI and round points represent treatments with *D. basalis*.

4.3.2. Wasp responses

The behaviour and development of *D. basalis* were not affected by the morph of the offspring's parents (Figure 4.2; Table 4.3 & Table 4.4).

4.3.3. Effect of the parasitoid wasp on weevil responses

The presence of *D. basalis* affected most weevil offspring response variables, with some differential effects between the offspring of flight vs. flightless parents (Figure 4.1; Table 4.1 & Table 4.2). Wasps reduced offspring *population sizes* from both flight and flightless parent weevil morphs (Figure 4.1A; Table 4.2), and reduced *larvae survival* for offspring produced by the flight morph but not for the offspring of flightless morph parents (Figure 4.1B; Table 4.2). When *D. basalis* were present, *C. maculatus* offspring from both parent morphs took longer to develop into adults (Figure 4.1C; Table 4.1). Since the larval stages are those that consume and damage the bean, longer development of offspring turning into adults resulted in higher *total bean loss* and *per capita bean loss* in the presence of wasp, particularly for offspring from flight morph parents (Figure 4.1A & B; Table 4.2). Bean loss in treatments (mean and SD = 8.45% ± 1.23% of the original bean mass) largely reflected damage caused by the weevil, as we found a small, estimated *water loss* of 0.25% ± 0.09% (mean and SD per Petri dish) of the original bean mass, on the control beans. The presence of wasps (which affect the larval stage) did not alter *adult lifespan* of offspring from either flight or flightless parents (Figure 4.1D; Table 4.1).

Table 4.3 Anova outputs for linear mixed effects regression models predicting *D. basalis* responses *development period* and *lifespan*) as a function of the main predictors: *C. maculatus* parent morph (flightless or flight). Some models also included as predictors: *C. maculatus* egg number per eppendorf, eppendorf ratio of flightless offspring, eppendorf *D. basalis* count. We report the sum of squares (sum sq), the arithmetic mean (mean sq), degrees of freedom in the numerator (numDF), degrees of freedom in the denomenator (denDF), F-value and P-value for variables. Interaction terms are indicated by two variable names separated by a colon. Significant variables and interactions are highlighted in bold.

Variable	Sum sq	Mean sq	NumDF	DenDF	*F*-value	*P*-value
Development period						
Parent morph	0.257	0.257	1	40.479	0.544	0.465
Eppendorf no eggs	0.213	0.213	1	43.291	0.450	0.506
Eppendorf percent flightless	0.725	0.725	1	38.587	1.533	0.223
Eppendorf wasp count	0.098	0.098	1	29.517	0.207	0.653
Adult lifespan						
Parent morph	0.192	0.192	1	141.000	0.243	0.623
Eppendorf no eggs	0.052	0.052	1	141.000	0.065	0.799
Eppendorf percent flightless	1.475	1.475	1	141.000	1.863	0.175
Eppendorf wasp count	1.567	1.567	1	141.000	1.980	0.162

Table 4.4 Anova outputs for generalized linear mixed effects regression models predicting *D. basalis* responses *population size*, *drumming behaviour* and *ovipositing behaviour* as a function of the main predictors: *C. maculatus* parent morph (flightless or flight). Some models also included as predictors: *C. maculatus* egg number per Petri dish replicate, ratio of flightless offspring per Petri dish replicate, and the mean number of *C. maculatus* eggs per Petri dish replicate. We report Chi-square statistic (Chisq), degrees of freedom (DF) and P-value. Interaction terms are indicated by two variable names separated by a colon. Significant variables and interactions are highlighted in bold.

Variable	Chisq	Df	*P*-value
Population size			
Parent morph	0.632	1	0.427
Petri no eggs	0.000	1	0.995
Drumming behaviour			
Parent morph	0.059	1	0.808
Petri mean no eggs	0.059	1	0.808
Ovipositing behaviour			
Parent morph	2.657	1	0.103
Petri mean no eggs	0.001	1	0.983

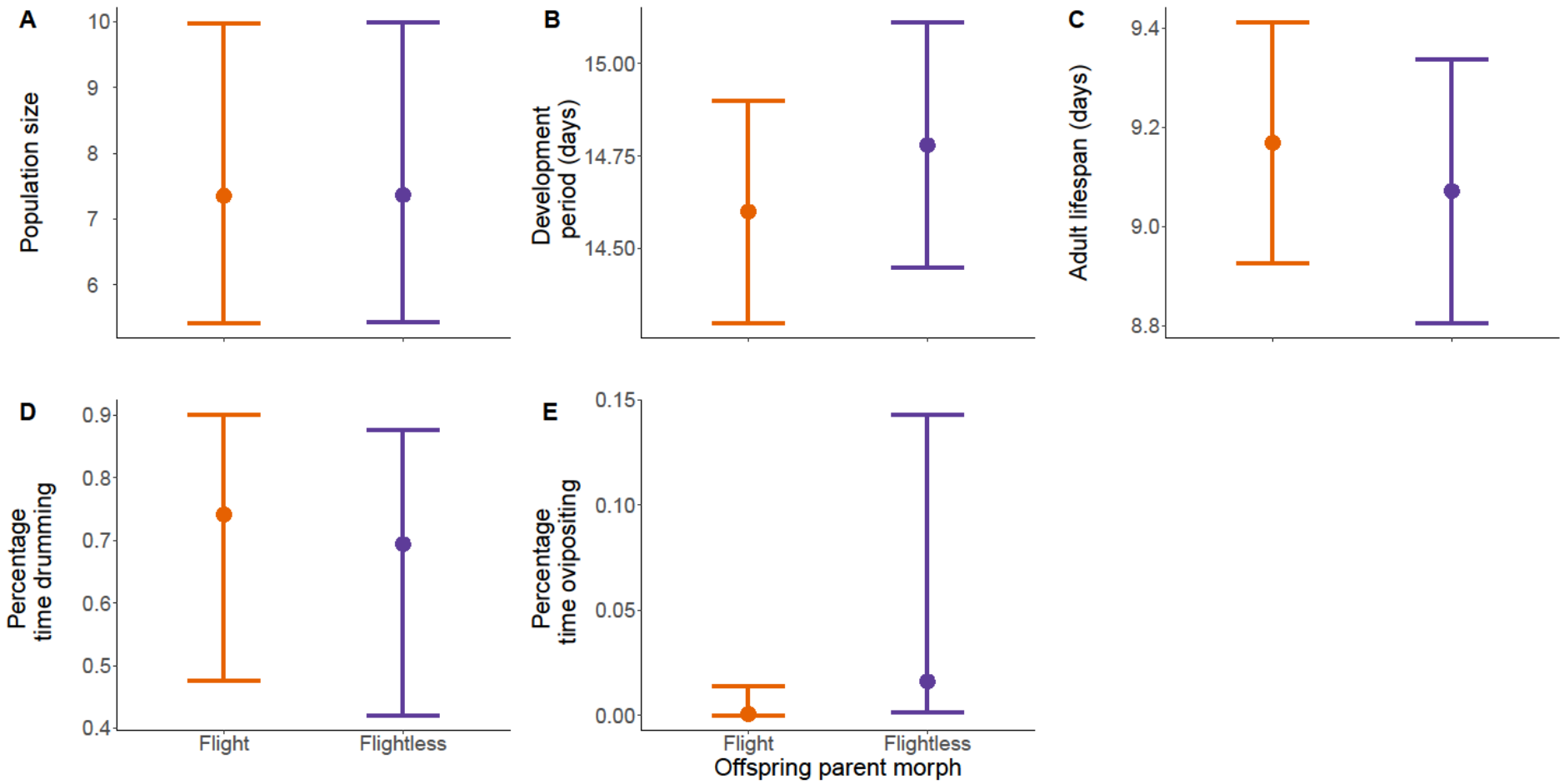

Figure 4.2 Parasitoid wasp *D. basalis* performance on the offspring of flight and flightless *C. maculatus* parents. We show the estimated model mean (symbol) and 95% confidence intervals (lines) for each treatment.

4.4. Discussion

Dinarmus basalis has been proposed as a biological control for *C. maculatus* (Ketoh et al., 2002) but to ensure its effectiveness it is important to consider how this wasp affects and is affected by the two described *C. maculatus* morphs (and their offspring), which differ in morphology and life history traits. Our study addressed this question showing that *D. basalis* can affect the offspring of either morph (with some differences) and does not respond differently to offspring produced by either flight or flightless parents.

4.4.1. Variation between offspring of weevil morphs

Previous work has found differences between the flight and flightless morphs of *C. maculatus* (Caswell, 1960; Utida, 1972). In the present study, we found that the *offspring* of *C. maculatus* from different parent morphs (flight and flightless) also perform differently, potentially influencing the impact as pests of each morph. For example, adult lifespan was longer in offspring that came from flight parents and individuals that were morphed as flight, which agrees with previous literature (adults of the flight morph can live twice as long as flightless morph adults; Appleby and Credland, 2001). Having longer lifespans can enable individuals to disperse over longer distances and potentially infest bean storages in a wider range. While morph types tend to develop under different environmental conditions, our results suggest partial heritability of morph type, as previously suggested by Caswell (1960) and Utida (1974).

We also found that offspring, from both flight and flightless parents, which were classified as flight morphs, had longer development periods from egg to adult emergence than offspring classifies as flightless morph (mean and SD = 61.74 ± 2.15 days and 59.82 ± 2.48 days for flight and flightless morphed offspring, respectively); which agrees with the

longer development period of flight morphs described by Caswell (1960) and Utida (1972). Both authors agreed that the traits seen in flight and flightless forms are suited for dispersal and storage respectively, although they did not detail why, however, it is likely due to development of the wings in the dispersal morphs. When we compared the average development period of offspring from flight vs. flightless parents, intriguingly it was the flightless parents (who produced more flightless morph offspring which had shorter development periods) who produced offspring with the longest average development periods (Table 4.1C; Table 4.1). Our results, could reflect trade-offs between competition and dispersal (Burton et al., 2010): flight morphs are dispersers more likely to colonize new areas, therefore, despite longer development period allowing more time to develop an individuals' wings, faster developing offspring *on average* would be beneficial to ensure a population is established if the resource continues to be available or to ensure adults are produce if the resource is more ephemeral. Future experiments are needed to explore these potential trade-offs.

Another result which is contrary to the literature (Utida, 1972) was the difference in fecundity among morphs; flight parents produced more eggs than flightless parents. The discrepancy likely reflects the fact that we assessed very short-term fecundity (eggs laid within a 24h period) whereas previous work had focused on lifetime fecundity. These differences again have potential implications for pest impacts. Our results suggest dispersing weevils (flight morphs) may produce many eggs quickly to establish a population, which is then maintain in the longer term by greater lifetime fecundity described in the literature of the established flightless morph.

4.4.2. Wasp responses

Host variation has been shown to affect parasitoid fitness (Urrutia et al., 2007). We therefore hypothesised that life-history, behaviour and biochemical differences between

the offspring from the two weevil morphs, such as water and crude fat content (Nwanze et al., 1976; Utida and Takahashi, 1958) could affect the development of *D. basalis* and its effectiveness as a biocontrol. Morph-specific effectiveness could lead to different control potential for different types of outbreaks; more recently affected bean storages are likely to be infested by the flight morph, whilst in older more established infestations, the flightless morph is likely be more prevalent. Counter to our prediction, *D. basalis* performance and behaviour was found to be similar, regardless of whether *D. basalis* was parasitising offspring from flight or flightless parents. Biochemical differences between weevil morphs may not have affected the wasp because, as seen with most parasitoids, *D. basalis* are smaller in body size compared to their host and thus, both morphs may provide enough food and nutrition for *D. basalis* development. Indeed, even if parasitoids prefer optimal size hosts, they can still successfully parasitise and grow on hosts of varying sizes (Cohen et al., 2005; Morris and Fellowes, 2002). Moreover, some parasitoids, like the females of the biological control parasitoid *Aphidius colemani*, show no preference between winged and wingless morphs of the aphid *Myzus persicae*, and in no-choice experiments (similar to ours) had similar performance with both morphs (Pirotte et al., 2018). Additionally, whilst offspring parental morph did not affect *D. basalis* performance, we did find more adult wasps emerging from beans that had more *C. maculatus* eggs, which could reflect the benefits of reduced parasitoid competition (Mayhew and Van Alphen, 1999).

4.4.3. Effect of the parasitoid wasp on weevil responses

An effective biological control should reduce the pest population such that damage remains below a certain economic threshold (Pappas et al., 2017; Stiling and Cornelissen, 2005). We found clear positive effects of *D. basalis* as a natural enemy and biological control, despite only two wasp females only being allowed to oviposit for 24h in our study. Within this short time period, *D. basalis* reduced *C. maculatus* offspring

population size from both parent morphs by 22.98% and lowered 15.23% of larval survival of offspring from flight parents, compared to 0.63% decrease of larval survival of offspring from flightless parents in the presence vs. absence of the wasp. Reduced population size has been described in previous studies (Amevoin et al., 2007; Ketoh et al., 2009; Sanon et al., 1998), and here we show it can occur in population started by either weevil morphs. Additionally, the lowered survival in flight parent offspring may reflect *D. basalis* preference to flight offspring larva as we predicted. Flight adults have higher crude fat and biomass compared to flightless adults (Chaudhuri, 2005; Utida, 1972), and therefore it is likely that these traits could pass to their offspring (even when they are in their larval stage). If this is the case, parasitising flight offspring would be more beneficial than parasitising flightless offspring, although we did not find any change in performance of *D. basalis* offspring emerging from the offspring of flight vs. flightless parents. Future studies comparing the fat content of offspring to their parent for both flight and flightless morphs, as well as weighing the emerged wasps from flight and flightless treatments, may help confirm this.

Out of all the studied *C. maculatus* performance traits, adult lifespan was the only variable unaffected by the presence of *D. basalis*. *Dinarmus basalis* is an ectoparasite which feeds on the larval stage of its host (Qumruzzaman and Islam, 2005; Sankara et al., 2014), and so larvae parasitised and affected by *D. basalis* are unlikely to survive to adulthood. Our findings show that neighbouring, uninfected *C. maculatus* larvae that developed into adults lived just as long as those in no wasp treatments, thus suggesting that they were unaffected by wasp presence. However, we did not test for possible impacts on fecundity or behaviour of these adults, so it is possible that wasp presence can affect weevils in some way even if not directly parasitised.

Whilst the presence of *D. basalis* reduced the number of weevils emerging, the amount of bean loss (overall or per capita) was not reduced, and instead suggested a potential

negative impact: the offspring of the flightless morph consumed the same amount of bean despite the presence of the wasp, and in the case of offspring of the flight morph, more bean was actually lost. The lack of reduced bean loss by the biocontrol may be due to parasitized larvae increasing their bean consumption to sustain the parasitoid, or an indirect consequence of the slower weevil development found in the presence of wasps. It is the larval stage of *C. maculatus* which consumes the bean (Caswell, 1960), and therefore slower development rates could lead to higher bean consumption. Other ectoparasitoids like *Dendrocerus carpenteri* has also been found to delay host development, altering the biochemistry and physiology of their hosts to prevent successful host pupation (Beckage and Gelman, 2004). While we found no benefits in terms of less bean loss in the short term (and even had potential negative impacts with flight morphs), the reduction in population size and the lower survival for the flight morph *D. basalis* could over time compensate and result in effective control, if the total number of weevils and survival of dispersing weevils decrease. Future experiments run over multiple generations are necessary for assessing the effectiveness of this potential trade-off over time. For the short term, exploration of integrated pest management with natural oils, such as neem, could help minimize the negative effects of the short-term loss in bean.

4.4.4. Future management strategies

Since the flight morph is considered the 'dispersal morph (Messina, 1987) our results suggest that *D. basalis* could be particularly effective in preventing or reducing *C. maculatus* infection of nearby stores by limiting the survival of the flight morph. The parasitoid *Aphidius colemani* has also been found to reduce the winged morph of the aphid *Myzus persicae* (akin to the flight morph in *C. maculatus*), however only if *M. persicae* is parasitised in its third instar (Pirotte et al., 2018). Other parasitoids have been known to prefer certain phenotypes or phenotypic traits within their host species. For

example, the ectoparasitoid *Sclerodermus harmandi*, prefers to parasitise larger *Monochamus alternatus* larvae, as their offspring consequently have larger survival and body weight compared to parasitising smaller larvae (Liu et al., 2011). This behaviour occurs despite a lower parasitism rate and adult mortality as larger *M. alternatus* larvae are likely harder to parasitise (Liu et al., 2011). In our study, wasp behaviour and performance did not differ between the morphs, but we did find trade-offs with flight morphs being less likely to survive and yet more likely to cause bean loss.

Since there are several other known parasitoids of *C. maculatus* (Van Huis et al., 2002), future work investigating the effects of *C. maculatus* morph on the performance of these other parasitoids, may enable farmers to develop more effective pest management strategies. For example, if further research finds different parasitoids perform better on specific *C. maculatus* morphs, management strategies could focus on the most effective parasitoid if a morph was dominant or depending on the stage of infestation or use different parasitoids combined or in sequence to provide better, longer lasting control. Our results suggest *D. basalis* is more effective at controlling the flight morph (although not its immediate bean damage), therefore *D. basalis* may be more effective to prevent initial establishment. However, future experiments are needed to determine whether the results seen in controlled laboratory conditions are maintained under the different and/or fluctuating environmental conditions seen in the field; especially in terms of temperature and humidity, which determine *C. maculatus* morph the life histories and development of parasitoids.

4.4.5. Conclusions

Dinarmus basalis impacted and performed similarly on the offspring of both *C. maculatus* morphs. However, the wasp's overall effectiveness as a biological control partly differed between the two *C. maculatus* morphs, and in our short- term (one generation) study, *D.*

basalis was not effective at reducing the pest impact (bean loss). Our study provides experimental evidence that intraspecific variation within pest species can influence biological control effectiveness and emphasize the importance of considering this variation when trialling potential biological controls for pest species, especially for those displaying distinct polymorphisms such as species in the superfamily Aphidoidea.

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Appendix

Table 4A.1 Traits used to assess flight and flightless morphs on day of emergence based on studies by Caswell (1960) and Utida (1972) (see Figure 4A.2 for examples)

	Question	Options
1	Is the pygidium more tucked into the elytra than outside of the elytra?	Yes - Flight
		No - Flightless
		Unsure – go to 2
2	What is the body shape?	More round/ triangular - Flight
		More ellipsoidal - Flightless
3	Looks dark ‘wholly black’ ground colour	Yes - Flight
		No - Flightless

Table 4A.2 Traits used to assess flight and flightless morphs when individuals are older based on studies by Caswell (1960) and Utida (1972) (see Figure 4A.2 for examples).

	Question	Options
1	Is the pygidium more white/ gold than the weevil's ground colour (red/brown/black)?	Yes - Flight No - Flightless Unsure – go to 2
2	Does the *C. maculatus* have denser white/ gold hairs compared to the pygidium of a flightless morph?	Yes - Flight No - Flightless
3	What is the body shape?	More round/ triangular - Flight More ellipsoidal - Flightless
4	Is the pygidium more tucked into the elytra than outside of the elytra?	Yes - Flight No - Flightless
5	Looks dark 'wholly black' ground colour	Yes - Flight No - Flightless
6	Is the pygidium more pointed than round?	Yes - Flightless No - Flight
7	Is the pygidium noticeably black with a thin white stripe (as opposed to all one colour)?	Yes - Flightless No - Flight

Table 4A.3 Summary of sample sizes and treatments in the study

Treatment	Number of Petri dishes/ repeats	Total number *C. maculatus* at start of experiment	Total number of *D. basalis* used for each treatment
Just Bean (control)	15	NA	NA
Flight Morph	10	310 virgin flight *C. maculatus* (250 F, 60 M) were used to create a 'flight morph mating dish'. Six females were then used per Petri dish for oviposition	NA
Flightless Morph	10	310 virgin flightless *C. maculatus* (250 F, 60 M) were used to create a 'flightless morph mating dish'. Six females were then used per Petri dish for oviposition	NA
Flight Morph + *D. basalis*	10	Six females from the 'flight morph mating dish' were used per Petri dish for oviposition	20
Flightless Morph + *D. basalis*	10	Six females from the 'flightless morph mating dish' were used per Petri dish for oviposition	20

Table 4A.4 All *C. maculatus* models tested to analyse the effects of *C. maculatus* parent morph and presence of *D. basalis* on *C. maculatus* offspring performance: *bean loss*, *per capita bean loss, population size, larvae survival*, *development period*, *adult lifespan,* and additionally *C. maculatus*: *egg number, percentage flightless* and *offspring morph*. * indicates tested interactions and models used are highlighted in bold.

Model	Fixed predictors	Random Factors
Bean loss		
	Parent morph + presence of *D. basalis* + *C. maculatus* Petri count + *C. maculatus* Petri percentage flightless morph	NA
	Parent morph*presence of *D. basalis* + *C. maculatus* Petri count + *C. maculatus* Petri percentage flightless morph	**NA**
Per capita bean loss		
	Parent morph + presence of *D. basalis* + *C. maculatus* Petri percentage flightless morph	NA
	Parent morph*presence of *D. basalis* + *C. maculatus* Petri percentage flightless morph	**NA**
Population size		
	Parent morph + presence of *D. basalis* + *C. maculatus* Petri egg count	**NA**
	Parent morph*presence of *D. basalis* + *C. maculatus* Petri egg count	NA
Larvae survival		
	Parent morph + presence of *D. basalis*	**Petri dish**
	Parent morph*presence of *D. basalis*	Petri dish

Development Period		
	Parent morph + presence of *D. basalis* + *C. maculatus* eppendorf egg count	**Petri dish: Eppendorf name**
	Parent morph*presence of *D. basalis* + *C. maculatus* eppendorf egg count	Petri dish: Eppendorf name
Adult lifespan		
	Parent morph + presence of *D. basalis* + *C. maculatus* eppendorf egg count + *C. maculatus* emergence morph	**Petri dish: Eppendorf name**
	Parent morph*presence of *D. basalis* + *C. maculatus* eppendorf egg count + *C. maculatus* emergence morph	Petri dish: Eppendorf name
	Parent morph**C. maculatus* emergence morph + presence of *D. basalis* + *C. maculatus* eppendorf egg count	Petri dish: Eppendorf name
Egg number		
	Parent morph	**NA**
Percentage flightless		
	Parent morph + presence of *D. basalis* + *C. maculatus* Petri dish egg count	**NA**
	Parent morph*presence of *D. basalis* + *C. maculatus* Petri dish egg count	NA
	Parent morph**C. maculatus* Petri dish egg count + presence of *D. basalis*	NA
Offspring morph		
	Parent morph + presence of *D. basalis* + scale(*C. maculatus* eppendorf egg count) + scale(*C. maculatus development period)* + scale(*C. maculatus adult lifespan)*	**Petri dish: Eppendorf name**
	Parent morph*presence of *D. basalis* + scale(*C. maculatus* eppendorf egg count) + scale(*C. maculatus development period)* + scale(*C. maculatus adult lifespan)*	Petri dish: Eppendorf name

Parent morph*scale(*C. maculatus development period)* + presence of *D. basalis* + scale(*C. maculatus* eppendorf egg count) + scale(*C. maculatus adult lifespan)*	Petri dish: Eppendorf name

Table 4A.5 All *D. basalis* models tested to analyse the effects of *C. maculatus* parent morph on parasitoid performance: *population size*, *development period*, *drumming behaviour*, *ovipositing behaviour* and *adult lifespan*. * indicates tested interactions and models used are highlighted in bold.

Model	Fixed predictors	Random Factors
Population Size		
	Parent morph + *C. maculatus* eppendorf egg count	**Petri dish**
	Parent morph**C. maculatus* eppendorf egg count	Petri dish
Development Period		
	Parent morph + *C. maculatus* eppendorf egg count + *D. basalis* eppendorf count + *C. maculatus* eppendorf percentage flightless morph	**Petri dish: Eppendorf name**
	Parent morph**C. maculatus* eppendorf egg count + *D. basalis* eppendorf count + *C. maculatus* eppendorf percentage flightless morph	Petri dish: Eppendorf name
	C. maculatus eppendorf egg count**D. basalis* eppendorf count + Parent morph + *C. maculatus* eppendorf percentage flightless morph	Petri dish: Eppendorf name
	Parent morph**D. basalis* eppendorf count + *C. maculatus* eppendorf egg count + *C. maculatus* eppendorf percentage flightless morph	Petri dish: Eppendorf name
Drumming behaviour		
	Parent morph + *C. maculatus* Petri dish egg count	**Petri dish**
	Parent morph**C. maculatus* Petri dish egg count	Petri dish
Ovipositing behaviour		
	Parent morph + *C. maculatus* Petri dish egg count	**Petri dish**

	Parent morph*_C. maculatus_ Petri dish egg count	Petri dish
Adult lifespan		
	Parent morph + _C. maculatus_ Petri dish egg count + _C. maculatus_ eppendorf percentage flightless morph	**Petri dish**
	Parent morph*_C. maculatus_ Petri dish egg count + _C. maculatus_ eppendorf percentage flightless morph	Petri dish
	Parent morph*_C. maculatus_ eppendorf percentage flightless morph + _C. maculatus_ Petri dish egg count	Petri dish

Table 4A.6 Anova outputs for linear regression models predicting cowpea weevil *C. maculatus* responses *egg number* and *percentage flightless* as a function of the main predictors: parent morph (flightless or flight). *Percentage flightless* also included the predictors: presence of *D. basalis* (yes or no) and *C. maculatus* egg number per Petri dish replicate. We report the sum of squares (sum sq), the arithmetic mean (mean sq), degrees of freedom in the numerator (numDF), degrees of freedom in the denomenator (denDF), F-value and P-value for variables. Significant variables and interactions are highlighted in bold.

Variable	Sum sq	DF	*F*-value	*P*-value
Egg number				
Parent morph	**21669.000**	**1**	**20.063**	**<0.0001**
Percentage flightless				
Parent morph	**1041.260**	**1**	**12.037**	**0.001**
Presence of wasp	177.740	1	2.055	0.160
Petri no eggs	233.190	1	2.696	0.109

Table 4A.7 Anova outputs for generalized linear mixed effects regression models predicting *offspring morph* as a function of the main predictors: *C. maculatus* parent morph (flightless or flight) and presence of *D. basalis* (yes or no), and also: *C. maculatus* egg number per eppendorf, *C. maculatus development period* and *C. maculatus adult lifespan.* We report Chi-square statistic (Chisq), degrees of freedom (DF) and P-value. Significant variables and interactions are highlighted in bold.

Variable	Chisq	Df	*P*-value
Parent morph	**34.8957**	**1**	**<0.0001**
Presence of wasp	1.1391	1	0.286
Eppendorf no eggs	**18.1779**	**1**	**<0.0001**
Weevil *development period*	**6.5734**	**1**	**0.010**
Weevil *adult lifespan*	**28.6874**	**1**	**<0.0001**

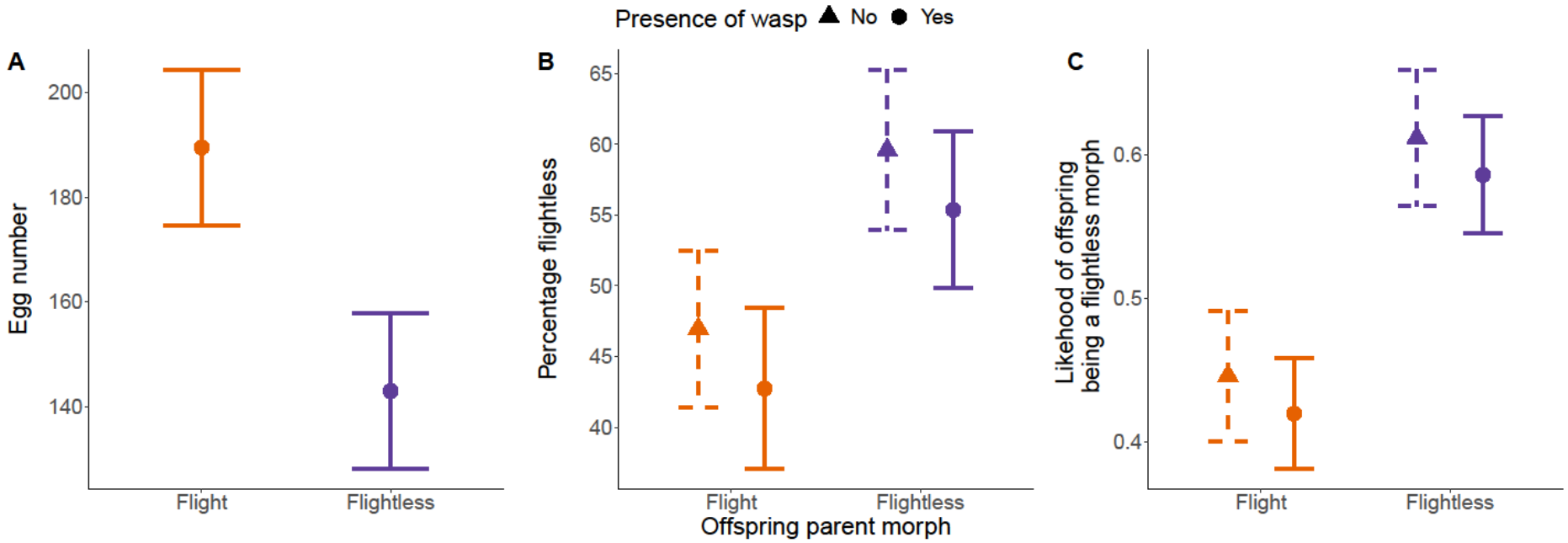

Figure 4A.1 Other *C. maculatus* performance traits observed. We show the estimated model mean (symbol) and 95% confidence intervals (lines) for each treatment. Dashed CI with triangle points represent treatments without *D. basalis*, and solid CI and round points represent treatments with *D. basalis*.

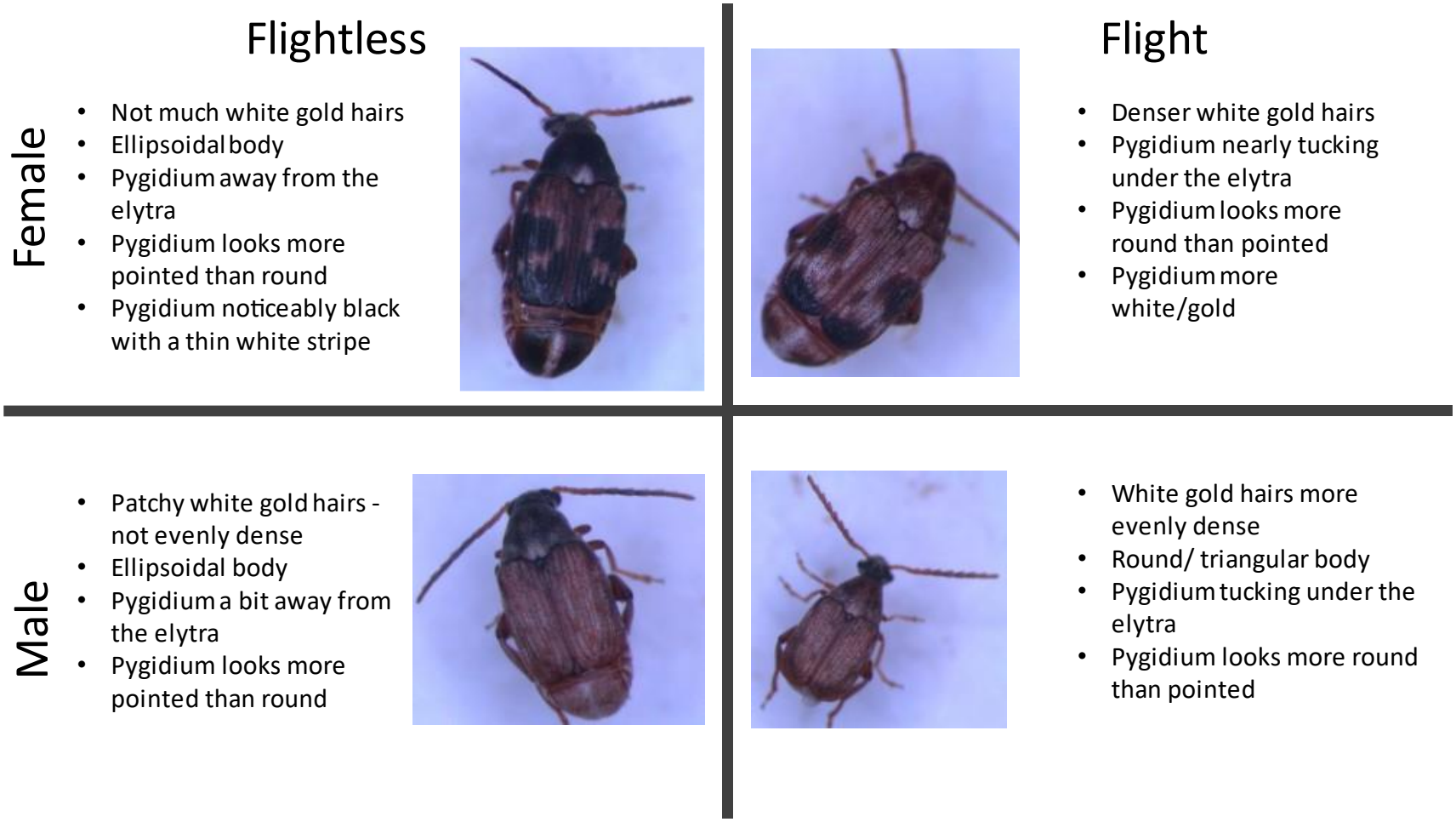

Figure 4A.2 Diagram with examples of flight and flightless *C. maculatus* (female and male). The text lists the traits which has helped identify whether the individual is flight or flightless (see Table 4A.1 & Table 4A.2 for list of traits).

CHAPTER 5. General discussion

CHAPTER 5. General discussion

5.1. Discussion

Previous studies have found that intraspecific variability can be important for species performance and ecological success, where more variable populations are more stable in their size and resilient to environmental change (Forsman and Wennersten, 2016). Although more controlled experimental studies researching intraspecific variation in ecology are starting to be conducted (e.g. Barbour et al., 2016; Butterfield and Wood, 2015; Raffard et al., 2017; Start, 2019; Start and Gilbert, 2017), more studies are needed, especially analysing effect in changing environments. Additionally, there is a need for more studies in taxa besides terrestrial vascular plants and freshwater fish (Des Roches et al., 2018). This thesis therefore set out to investigate how intraspecific variation influences population and individual processes: (1) on several insect species, (2) in controlled experimental studies, and (3) under varying environmental conditions to address these gaps within the literature. We looked at the effects of intraspecific variation on pest and invasive species to provide guidance for improved management including use of biocontrol agents. In the final experimental chapter, effects of intraspecific variation on multiple trophic levels were also analysed. The results from this thesis contribute to our understanding of the role of intraspecific variation on pest and biological control management.

5.1.1.1. Within populations

Phenotypic intraspecific variation *within* populations influenced responses in some species but not in others. In chapter 2, phenotypically variable and non-variable subpopulations for each of the pest *S. oryzae* strains tested performed similarly on all four environmental (grain) treatments. The variation in body size *within* an *S. oryzae* population may be largely due to phenotypic plasticity, and thus, parental variation may be less likely to influence the performance of the offspring under standardized conditions. On the other hand, in chapter 4, we showed that phenotypic intraspecific variation influenced the pest performance of *C. maculatus*: offspring from flight morph parents had shorter development periods and longer adult lifespan compared to offspring from flightless parents. Presumably, these traits allow offspring from flight morph parents to disperse faster and colonise new further resources that can be reached by longer living adults. Additionally, the biological control wasp, *D. basalis*, was more effective at reducing the survival of the dispersal (flight) morph. Intraspecific variation within populations is particularly noticeable in polymorphic species, where distinct and extreme morphs exist and may be associated with fitness trade-offs because different morphs may be best adapted to different niches (Wennersten and Forsman, 2012; Zera and Denno, 1997). High levels of variation, including polymorphism, are predicted to enable species to adapt to a wider range of conditions and changing resources (due to utilizing different resources in polymorphic species) compared to locally adapted less diverse populations (Benito Garzón et al., 2011; Forsman and Åberg, 2008; Oliver et al., 2015). However, if variation (direct trait variation or the potential for plasticity), is not heritable, its effect on population dynamics and ecological interactions would be less clear and more difficult to predict. If variation in a pest species influences its performance but cannot be manipulated or controlled, management may be limited, and yet

acknowledging the potential uncertainty in response would still be key to ensure a fair assessment of varying strategies.

The different effects of intraspecific variation, when looking at a potentially plastic trait in chapter 2 vs a polymorphic traits in chapter 4 results, support Wennersten and Forsman's (2012) review of intraspecific phenotypic variation studies. They found that the consequences of phenotypic plasticity worked on different time scales to that of polymorphism, due to the different mechanisms which produce each form of variation.

5.1.1.2. Between populations

While variation *within* populations was not always associated with changes in performance, our results found more consistent effects of intraspecific variation *between* populations, which was more likely to reflect genetic variation in our study species. In both chapters 2 and 3 we found differences in the performance of populations (strains) from *distinct* geographical regions. Additionally, for chapter 2, these differences in the performance of populations changed depending on the grain treatment. In contrast, the two stocks from the *same* geographical region in chapter 3, which differed in the time they had been lab reared (short term vs. long term), did not differ in their performance, even under the two environmental treatments tested (Fung et al., 2020). As often found in nature, individuals from geographically different populations are more distinct than those from the same geographical area (e.g. *Arabidopsis thaliana;* DeLeo et al., 2018), likely due to larger differences in the environment and geographical structures (Moran et al., 2016). This suggests that intraspecific variation among populations may be a more significant factor to be considered for pest and biocontrol management, as it is likely to have large consequences than variation within populations. The most frequent type of

biological control are inoculative or “classic” biological control (where natural enemies are taken from where the invasive pest was thought to originate from, and used as a biological control wherever the pest is accidentally introduced; DeBach, 1964; van Lenteren, 2012), and augmentative biological control (where more of the same natural enemies are added to an area, as the naturally occurring populations are not as effective or in low numbers; Perez-Alvarez et al., 2019; van Lenteren, 2012). Both types of biological control ignore potential intraspecific differences between and within populations of the same pest species, as only one strain/ population of natural enemy is mass-reared for pest management use (van Lenteren, 2012). There are some studies starting to look into intraspecific traits to improve classic biological control performance, for example, under different environmental conditions or to become compatible with other pest control methods such as pesticides (Bielza et al., 2020).

Additionally, we found that the presumably more genetically diverse hybrid populations did not consistently perform better than the other populations (strains) assessed under different environmental conditions. The hybrid strain in chapter 2 was one of best performing populations on some grain conditions, but in other grains, its performance was in between that of the other strains, which could reflect trade-offs (e.g., quality and quantity of offspring). Furthermore, the hybrid populations of *A. itadori* in chapter 3 did not improve biological performance, and instead produced negative effects (slower development rate and lower survival from egg to adult) under both the environmental conditions assessed; potentially due to outbreeding depression, which has been seen in previous studies (Edmands, 1999; Hwang et al., 2011; Peer and Taborsky, 2005). With the uncertain effects of intraspecific variation seen in our findings, our work supports the need for further exploration.

5.1.1.3. Summary of findings

Results from this thesis shows there is no unique answer to the question of whether or how intraspecific variation alters the population dynamics and interactions of invertebrate pests and biocontrols. Given this uncertainty, we consider the cautionary approach should be for studies testing different strategies for pest management and biological control, to explicitly consider and test the potential role of intraspecific variation in performance and impact. Previous work based on single type individuals could be used as a baseline 'recipe' to be modified, following additional research to examine performance when considering distinct individuals and populations.

5.1.2. General limitations and directions for future research

5.1.2.1. Multiple generations

Due to time constraints, the studies within the thesis were only conducted for one generation. However, the performance of a species and the effects of intraspecific variation may change over generations. A meta-analysis by Yin et al., (2019) shows that transgenerational effects can vary by environment, taxonomic group, and trait. If time and costs allow intraspecific studies on pest or biological control species should ideally be performed over multiple generations to assess multigenerational effects. Additionally, cross-generational effects can also be sex dependant, as seen in chapter 2 and other previous studies (Hellmann et al., 2020), and this should be considered, although sex-effects are unlikely to be as large as the overall multigenerational effects.

5.1.2.2. Multiple traits

Future studies should also consider exploring the effects of variation over different traits within a species. In chapter 2 we explored the effect of variation in the trait body size; an important trait which correlates to reproductive traits for many taxa (Shine, 1988). For example, larger reptiles, annual plants and terrestrial insects are generally more fit compared to their smaller conspecifics, having greater survival, fecundity and mating success (Kingsolver and Huey, 2008). Size has also been shown to be correlated in mammals with population processes likes extinction risk and establishment (González-Suárez et al., 2015; González-Suárez and Revilla, 2013). However, there are multiple traits (behavioural and morphological) which show intraspecific variation, including attack rate, competitiveness, development time, colouration, foraging behaviour, thermal adaptation and pesticide resistance (see Bolnick et al., 2011; Forsman et al., 2020; Lirakis and Magalhães, 2019). Additional work is needed to understand the role of variation in morphological vs ecological and behavioural traits. Of particular interest would be to evaluate functional traits that play a role in the functioning and structure of ecosystems, such as tolerance to extreme temperatures, resistance to desiccation and the ability to disperse for arthropod biological controls (see Bielza et al., 2020).

5.1.2.3. Multiple environmental conditions

How intraspecific variation influences performance under different, and varying, environmental conditions should also be further explored. In chapter 3 we looked at biological performance under two different temperature and humidity conditions, which differed between treatments but were constant during the experiment. Given more time and quarantine greenhouse space, we would have liked to study the biological control's

performance under multiple, and potentially also variable within treatment, environmental conditions (especially those predicted under future climate change). This information will be needed to make sure that potential biological controls are 'future proof' (i.e. able to perform under current and predicted future conditions). Such studies could also reveal how well the proposed biological control for Japanese knotweed (or other species) will cope in the different seasons in the UK, explore times like winter when some insect species, including psyllids, hibernate (Hodkinson, 2009). Studies have shown that the effects of intraspecific variation are generally strongest in natural conditions (Forsman, 2014) and, as seen with *A. itadori*, the performance under lab conditions does not always translate to performance under field conditions (CABI unpublished results). Research under natural conditions is therefore a vital step to test to the validity of laboratory results and ensure effective pest management.

5.1.2.4. Intraspecific variation in different trophic levels

There is additional need to consider the consequences of intraspecific variation along trophic chains and its impact on species interactions. In chapter 4 we studied a system of three interacting species (bean, weevil and wasp), focusing on the consequences of intraspecific variation in a single species, the weevil, which displayed clear differences between morphs. Previous work has also considered the effects on variation on one trophic level (Sentis et al., 2020). For example Hughes et al., (2015) looked at tri-trophic system considering how intraspecific variation in the herbivore marsh peri-winkle *Littoraria irrorate* affected the structure and function of the surrounding plant community and of its common predator the crown conch*, Melongena corona*. As in our study, Hughes et al., (2015) and Sentis et al., (2020) only considered variation in one species. Future work to explore the potentially complex effects of systems in which all species exhibit measurable intraspecific variation would hopefully give us a fuller view of the

effects of intraspecific variation at the community and ecosystem level, especially the implications to ecosystem functioning, and may provide a better understanding of species co-existence for community ecologists, who have historically ignored this form of variation (Hausch et al., 2018). However, such studies would be complex and would need to involve: (1) communities where all species display intraspecific variation in at least one measurable trait, (2) communities which contain a few of species (~3-5), so that models and interactions are not too complex to interpret, and (3) ideally natural communities that have been well studied.

Farmers are also likely to use integrated pest management methods (e.g. also introducing natural pesticide neem or other natural parasitoids of *C. maculatus*), which would add more complexity to the system. If this is the case, the performance of all biological controls in the management scheme, on managing the two different *C. maculatus* morphs, would need to be further explored in the presence of the natural pesticide or parasitoid. Such studies would also allow the exploration of both intraspecific and interspecific variation effects in more complex communities.

5.1.2.5. Different taxa

We focused on looking at intraspecific variation in insect species as there were fewer studies in this speciose group (Des Roches et al., 2018). However, even when focusing on pest and biological control insect species within this thesis, we still saw mixed effects of intraspecific variation on population performance. There is therefore still a need to assess the effects on intraspecific variation in more taxa, to see whether there are any general effects common to particular groups of organisms or environmental conditions (Forsman and Wennersten, 2016). This gap could be bridged by firstly conducting an

updated meta-analysis, in line with those by Forsman and Wennersten (2016) and Des Roches et al. (2018). This approach could identify key taxa gaps (such as insects and amphibians), and also highlight gaps in current studies (such as collecting data on the traits survival *and* reproduction, and not just one or the other, to see the true impacts on population persistence; Paniw et al., 2021).

In regards to potential organisms to use for further experimental studies, we suggest starting with species from underrepresented taxa but increasingly being studied which display clear variation in traits (such as the California two-spot octopus, *Octopus bimaculoides* which displays variation in behaviour; Hofmeister, 2015), and species in which intraspecific variation can be used in an applied way. For the latter, other species used for management such as parasitic wasps to control aphids, or species which provide ecosystem services such as detritivores (e.g. terrestrial worms, woodlice, sea cucumbers etc) would be important focal groups. Species which are at risk or of need of conservation may also be of high priority, especially if they have smaller populations with a low amount of variation (e.g. white-headed langur, *Trachypithecus leucocephalus* and the Mauritius pink pigeon, *Nesoenas mayeri*). Intraspecific variation research on these species may help identify individuals or particular traits which can be used to 'buffer' at-risk populations, or identify subpopulations of at-risk species which need to be prioritised (Forsman, 2014; Mitchell et al., 2020).

5.1.3. Conclusions

The studies in this thesis have revealed that, for pests and biological controls, intraspecific variation can be important in some cases; however, it is still unclear if there are general circumstances or taxa for which it will be important often or conversely, could

be safely ignored. For example, phenotypic variation was important within populations of polymorphic species tested in chapter 4, but few effects were detected for pest populations tested in chapter 2. Additionally, chapters 2 and 3 found that genetic variation, among geographically separate populations, can have positive and negative effects on population performance and influence population performance in novel conditions.

More research is needed to gain a comprehensive understanding of the true effects of intraspecific variation on the dynamics and performance of different invertebrate species across varying ecosystems and environmental conditions. Until we know for certain for which systems and species intraspecific variation is important and can influence ecological interactions and ecosystem processes, we recommend ecologists explicitly acknowledge and consider variation among individuals in their studies.

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Appendix

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Effect of humidity and temperature on the performance of three strains of *Aphalara itadori*, a biocontrol agent for Japanese Knotweed

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ABSTRACT

Japanese knotweed (*Fallopia japonica*) is a highly damaging invasive species affecting UK infrastructure and biodiversity. Under laboratory conditions, the psyllid *Aphalara itadori* has demonstrated its potential to be a successful biocontrol agent for *F. japonica.* However, this potential has not materialised in the field where long-term establishment of *A. itadori* has been unsuccessful and faces the added challenge of climate change. Intraspecific variation (variation among individuals of a species) has been shown to support establishment in alien species and improve resilience to changing environmental conditions. Here we propose it could improve the performance of biocontrols. To test this possibility we compared the performance and impact on *F. japonica* of three strains of *A. itadori* with different genetic backgrounds, including a newly created hybrid. We hypothesize that genetic variability would be increased in hybrids resulting in greater biocontrol effectiveness (greater impact on plant growth). We also explored the potential influence of changing climate on performance, testing all strains under two humidity conditions (with the same temperature). Contrary to our expectation, the hybrid strain had the worst performance (slowest development rate and lower survival from egg to adult emergence) under both environmental conditions. Exposure to different strains of *A. itadori* did not result in consistent differences in plant growth, suggesting similar biocontrol effectiveness among strains. Under the drier, more stressful, conditions plants exposed to *A. itadori* had fewer leaves and accumulated less above-ground biomass. Overall, our results suggest that genetic variability may not be the key to improve *A. itadori* biocontrol effectiveness, but that predicted climate change, which anticipates drier and hotter summers in the UK, could reduce the growth potential of *F. japonica* when exposed to *A. itadori*.

1. Introduction

Invasive species are a significant problem in the United Kingdom, where they are estimated to cost the economy approximately £1.7 billion per annum (Booy et al., 2008; Williams et al., 2010). Invasive species are both damaging to the UK's infrastructure and to the native biodiversity. One of the most problematic invasive weeds in the UK is Japanese knotweed (*Fallopia japonica* (Houtt.) Ronse Decr.), a species native to Japan. The lack of fertile *F. japonica* males in Britain, as determined from Random Amplified Polymorphic DNA (RAPD) analysis, suggests that all *F. japonica* in the UK are derived from a single clonal individual that has reproduced through vegetative propagation (Hollingsworth and Bailey, 2000). This low genetic diversity however, has not hindered its invasive ability. *Fallopia japonica* has become established in a wide-range of habitats, and grows asexually from small fragments of underground root networks – rhizomes – sometimes weighing less than a gram (Bashtanova et al., 2009; Hollingsworth and Bailey, 2000). These features, as well as its rapid growth rate, make *F. japonica* highly invasive in the UK.

There have been varying attempts to locally eradicate or control *F. japonica*. Manual or chemical removal can work at a local scale; however, the costs and time requirements make these methods unfeasible as long-term or large-scale management solutions. Herbicide use in parks

Abbreviations: LTLR, long-term laboratory-reared strain; STLR, short-term laboratory-reared; SDI, Saturation Deficiency Index

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and riparian areas where the plant is most prevalent has become less acceptable (Forman and Kesseli, 2003). Classical biological control is often proposed as an effective alternative method for invasive species, such as *F. japonica*. Reuniting an introduced weed with its host-specific natural enemies from their country of origin has resulted in successful suppression of many invasive weeds worldwide (Clewley et al., 2012; Schwarzländer et al., 2018). In comparison to other control methods, biocontrol can be used everywhere and is generally cost effective and environmentally friendly (Wittenberg and Cock, 2001).

The use of biocontrol agents for *F. japonica* in the UK has been explored by the non-profit organisation CABI, UK, since 2003. Initially, candidate species were identified from the Kyushu Island of Japan, the region from where the UK invasive *F. japonica* clones are thought to have originated (Djeddour and Shaw, 2010). Out of the 186 candidate arthropod species considered, *Aphalara itadori* Shinji (Hemiptera: Aphalaridae), otherwise known as the Japanese knotweed psyllid, was found to be the best agent, since laboratory studies showed it to be host-specific (i.e. not affecting native plants) and highly damaging to *F. japonica*. However, despite its effectiveness under laboratory conditions (Grevstad et al., 2013), the establishment of viable populations in the field has been largely unsuccessful. A possible explanation for why field releases have failed is a lack of genetic and phenotypic variability in the batches of *A. itadori* that were released. Genetic bottlenecking is commonly implicated in the establishment failure of biocontrol agents (see review by Fauvergue et al., 2012). It is not unusual in biocontrol programs for host-range testing for specificity and safety to require a long period of laboratory rearing. Indeed, in the UK, *A. itadori* was maintained in the laboratory from 2004 until its approval for release in 2010 (Shaw et al., 2009). Because the released *A. itadori* came from populations maintained under Japanese summer conditions at 22 °C 13:11 h day:night 50–85% humidity for at least six years (~66 generations), they may have become conditioned to the controlled environment room, as well as having potentially lost genetic diversity. This 'colony effect' of laboratory reared animals has been seen in other insect species, such as in *Drosophila* when undergoing laboratory selection experiments (Harshman and Hoffmann, 2000) and when comparing wild to laboratory cultures of *Drosophila* (Sgrò and Partridge, 2000), and also in *Anopheles gambiae* (Huho et al., 2007). As a result, the long-term laboratory-reared *A. itadori* could have been ill-prepared for dealing with the variability in the natural environmental conditions in the UK.

Intraspecific variation – the diversity of characteristics amongst individuals of a species (Cianciaruso et al., 2009) – can be an important factor aiding in the establishment of alien species (Forsman, 2014), but as mentioned above, variability may be reduced in laboratory-reared organisms. Plant and animal species with higher levels of intraspecific genetic and phenotypic variation are more likely to establish successfully in new environments under laboratory, semi-natural and natural conditions, with the largest effects seen in natural experiments (Forsman, 2014). In addition, intraspecific variability can provide resilience to changes in climatic conditions (Reusch et al., 2005; Sgrò and Hoffmann, 2004). Under climate change, more variable populations are predicted to have an increased chance of containing individuals with genotypes that allow population persistence (Oliver et al., 2015) whereas locally adapted, less diverse populations are vulnerable because they have evolved traits to suite only local stress factors (Benito Garzón et al., 2011).

The establishment of *A. itadori* may also have been affected by the interaction of different climatic conditions. Hodkinson (2009) and pilot field experiments (CABI, unpublished data) have shown that *A. itadori* population dynamics, and therefore their potential for establishment in the UK, can be affected by rising temperatures and declining relative humidity. In the UK, under climate change, conditions are likely to become more stressful due to a predicted increase in temperature and decrease in humidity in the spring and summer (Murphy et al., 2010) when *A. itadori* are most active after hibernation (Hodkinson, 2009). Therefore, effective biocontrol requires consideration of how different environmental conditions could affect effectiveness and resilience to future changes in climate.

For this study we compared the performance of the strain used in historic biocontrol releases to two other strains with different genetic backgrounds. The first genetically different strain we tested was from the same locality as the original strain (Kyushu in South Japan) but had a shorter laboratory-rearing history (2 years compared to 13 years). Using a newly collected wild type strain would have been desirable but was not possible due to the timing and cost of a new collection and quarantine space. The second genetically different strain tested was a new hybrid strain created from two distinct provenances of *A. itadori*. To create the hybrid we combined males from Kyushu and females from Hokkaido (North Japan; Grevstad et al., 2013). The Kyushu and Hokkaido strains of *A. itadori* are genetically distinct and both strains, as well as the hybrid, can be distinguished using neutral molecular markers (Andersen et al., 2016). We tested a hybrid as a potential approach to increase genetic variability and vigor (Birchler et al., 2006; Szűcs et al., 2012). However, hybridization can also have negative effects which could reduce the potential of this new hybrid strain (Heinze et al., 2019; Peer and Taborsky, 2005). The performance and impact on *F. japonica* of the three strains was tested under two environmental conditions that reflected standard laboratory growing conditions and a drier environment reflective of climate change predictions.

2. Material and methods

2.1. Aphalara itadori strains

We used three *Aphalara itadori* strains. Two, the LTLR and STLR strains, were established using adults collected from Kyushu, Japan (in 2004 and 2015 respectively). The hybrid strain was created by mating LTLR strain males with females from a *A. itadori* line collected in 2007 in Hokkaido, Japan and reared since that date at the Agriculture and AgriFood Centre (AAFC) in Lethbridge, Canada. The crossing of lines was completed in December 2016 at AAFC-Lethbridge under 16L:8D laboratory conditions. Second generation adult hybrids (n ≈ 200) were shipped to the UK and reared in CABI under standard laboratory conditions (see below). We used fourth generation hybrids for oviposition during the experiment. All three strains were reared on knotweed in 100 × 90 × 100 cm Perspex cages (average ± SD: 16.9 °C ± 3.8 °C, 47.2% ± 10.7% RH and 14L:10D) in CABI's Egham quarantine greenhouse facility.

2.2. Experimental design and conditions

We tested two environmental conditions that we then characterized using empirical estimates of Saturation Deficiency Index (SDI), a measure of climate severity (Samways, 1987). In its simplest form, SDI is the difference between the saturation vapour pressure (SVP) at maximum temperature, and the actual vapour pressure of a volume of air at maximum temperature (Green and Catling, 1971; Samways, 1987). The value of SDI increases with rising temperature and/or decreasing relative humidity. For our experiment, treatments were created by changing humidity within experimental cages. Plants under high SDI conditions, reflective of climate change predictions (hotter and drier), had dry capillary matting for the base of the cage and a 40 × 50 cm gauze covered hole at the back of the cage to increase ventilation. Plants in low SDI conditions had wet capillary matting for the base of the cage, watered with 800 ml tap water every week, reflecting the standard laboratory growing conditions. We calculated empirical SDI values for each treatment cage following Abtew and Melesse, (2013) and Samways (1987):

$$SDI = \mathrm{SVP}\left(\frac{100 - RH}{100}\right) \quad (1)$$

where RH is relative humidity, and SVP is saturation vapour pressure

calculated based on temperature (T) as below:

$$SVP = 0.611e^{\left(\frac{17.27 \times T}{T+237.7}\right)} \quad (2)$$

Humidity and temperature were recorded during the experiment at 30-minute intervals using LogTag Haxo-8 dataloggers placed inside the sleeve of one randomly selected plant per cage. We estimated SDI using the humidity and temperature recorded at each 30-minute interval. For each day we then identified the three highest SDI values and calculated the arithmetic mean per cage of those maxima over the duration of the experiment. This resulted in six SDI values (one per cage). We averaged the three highest values instead of using the single highest value to control for potential outliers. There are alternative methods of calculating SDI (see Green and Catling, 1971), but we found results were equivalent with all methods (Table A1, Fig. A1).

Fifty-five days prior to the start of the first experimental batch, the rhizomes of 71 young *F. japonica* of uniform genetic stock (collected from a single *F. japonica* patch with vegetative reproduction) were cleaned and wet rhizome weights for each plant were obtained (average ± SD: 75.85 g ± 36.06 g). Each rhizome was potted in an individual plastic pot (14.7 cm diameter) with a saucer (16.5 cm diameter) and left to grow in a greenhouse under natural conditions (average ± SD: 21.0 °C ± 4.5 °C, 51.6% ± 12.4% RH and 14L:10D).

All experimentation was performed in quarantine glasshouses (average ± SD: 21.0 °C ± 4.5 °C, 51.6% ± 12.4% RH and 14L:10D). Due to space constraints in the glasshouses, the experiment was completed in three sequential batches over four months. For each batch, 14–15 days before the start of the experiment, 18 plants were cut to the fourth node above ground on the main stem and first node from the stem on branches, with additional stems cut to ground level. This allowed us to standardise above-ground measurements of biomass. Cut *F. japonica* material was collected and frozen, and dry weights later obtained for before and after above-ground weight comparisons. Plants were then randomly assigned a *A. itadori* strain, and six plants from each strain were placed into designated chambers for up to 8 days with 150 *A. itadori* adults to allow oviposition (n ≈ 25 *A. itadori* per plant).

After the oviposition period, the total number of eggs per plant was counted by searching the top and bottom of all leaves and nodes using a hand lens. Plants with very high numbers of eggs were removed from egging chambers earlier to avoid high egg density variation across treatments (batch one: one STLR low SDI and one hybrid high SDI plant; batch two: one STLR low SDI plant). Egg counts are minimum estimates because total counts would have required damaging the plant, which would have prevented the experiment. We make the assumption here that the number of visible eggs is proportionally related to the total number of eggs. Plants were then randomly assigned to a low or high SDI treatment, resulting in three plant replicates per strain per treatment per batch (experiment total: n = 9 plant replicates per strain per treatment, total n = 54). We used 1 m long insect sleeves supported by bamboo hoops for each plant to prevent *A. itadori* from moving between plants (Fig. A2). Each plant was placed in a 16.5 cm diameter saucer and irrigated twice a week manually to ensure *F. japonica* survival irrespective of treatment. Total adult counts began 37 days after plants were placed in treatment cages. Emergent adults were counted and removed using a manual aspirator every 6–7 days for six weeks to allow all adults from the eggs laid prior to the experiment to emerge. Although the nymphal stages cause the most damage to plants (Djeddour and Shaw, 2010), accurately counting nymphs without removal is complicated, therefore we used adult counts to infer survival to adult emergence. After all adults were counted, we obtained wet weights of above ground and below ground plant biomass. Above ground plant material was then frozen and dry weights were later obtained.

2.3. Response variables: Aphalara itadori performance and plant growth

We used survival to adult emergence (henceforth referred to as '*A. itadori* survival') and development rates to assess *A. itadori* performance. *Aphalara itadori* survival was adjusted for initial egg density, and was calculated as $100 * \frac{Adults}{Eggs}$, where *Eggs* was the total number of eggs counted before moving the plants to the experimental treatments, and *Adults* was the total number of emerged adults counted over the entire experiment for each plant. *Aphalara itadori* development rate was evaluated by comparing the number of adults for each plant (expressed as percentage of the total), counted at 1, 2 and 3 weeks after the first adult emergence in each cage. Counts after week 3 were not considered to avoid counting second generation offspring emerging. One STLR plant from the low SDI treatment was removed as it had extreme adult *A. itadori* numbers emerging compared to initial eggs counted.

Due to space limitations in the quarantine glasshouses, we could not assess how SDI treatments affected plants without *A. itadori*. We evaluated impacts of *A. itadori* on *F. japonica* by measuring differences in above and below ground biomass, number of leaves and stem height. There was considerable variation in these traits between plants, thus, in the variables rhizome weight, maximum height and leaf number, we did not compare absolute growth but instead calculated relative growth as $100 * \frac{(Final - Initial)}{Initial}$, where *Final* was the measurement taken at the end of the experiment and *Initial* was the measurement before the start of the experiment. For the variable above-ground weight, the *Initial* was taken as zero (plants were potted as rhizomes, without above ground material), and the *Final* was calculated as the sum of the material that had been removed just prior to the experiment (to standardize plant size) and the remaining material at the end. Both were measured as dry weights. Plant material was wrapped in foil and placed into an oven at 70–90 °C for 48 h or until dried. As it was not possible to dry rhizomes before the experiment without killing the plant, change in below ground biomass was calculated using wet weights. The number of leaves was counted at the start and the end of the experiment. Stem height was measured using a ruler from soil level to the tallest standing point on the plant.

2.4. Data analysis

We evaluated the effect of strain and SDI on *A. itadori* survival, development and the four measurements of *F. japonica* growth using linear mixed effect regression models fitted with function 'lmer' from package *lme4* (Bates et al., 2015) in R version 3.4.3 (R Core Team, 2017). Table A2 lists the fixed and random effects considered for each model. In summary, all models included as a random factor the batch number (one, two or three) and, for *A. itadori* survival and development, also observer ID (authors CF and CP, and Kate Constantine contributed to egg counting). All models included SDI and strain as fixed predictors. In addition, models assessing plant growth included as covariates: total number of adults to control for variation in insect densities, and rhizome weight to control for initial plant conditions (except when modelling rhizome weight). Models of *A. itadori* survival also included the total number of eggs as a covariate. To model *A. itadori* development we used a B-splines analysis based on count week to allow for non-linear changes in development. We tested models with additive effects only, as well as with interactions between strain and SDI treatment. In the case of development, Week was also tested for interactions (Table A2). Models with interactions were only considered to be supported if interaction terms were significant (p-value < 0.05). We evaluated model assumptions (normality and heteroscedasticity) plotting residuals from tested models. We used post-hoc tests based on R function 'difflsmeans' and 'lsmeansLT' from package *lmerTest* (Kuznetsova et al., 2017) to contrast among strains.

3. Results

3.1. Aphalara itadori performance

Aphalara itadori survival varied among strains ($F_{2,\ 36.17} = 12.49$, $P < 0.001$, n = 18, 17 and 18 for LTLR, STLR and Hybrid strains respectively; Table 1, Fig. 1A). In particular, survival from egg to adult emergence was significantly lower in hybrids (predicted mean [95% confidence intervals]: 26.00% [10.99–41.01]) compared to LTLR (57.72% [41.86–73.57]) and STLR (54.79% [34.49–75.08]) strains, but LTLR and STLR did not differ ($P = 0.68$). SDI did not significantly affect *A. itadori* survival ($F_{1,\ 46.86} = 1.66$, $P = 0.20$), but survival was proportionally higher in plants with fewer eggs suggesting a density dependence effect ($F_{1,\ 46.82} = 7.98$, $P = 0.007$).

The proportion of adults emerging generally decreased from the first to the third week, with earlier emergence time under low SDI (higher humidity, $F_{1,\ 153} = 28.34$, $P < 0.001$; Table 1). The LTLR strain had the fastest development rates, with notable difference under high SDI, with the LTLR strain having peak emergence in the first week one compared to both the STLR and the hybrid strain which displayed peak emergence during the second week (Table 1, Fig. 1B). There was an interaction between STLR and SDI, with the majority of STLR adults emerging sooner under lower SDI ($F_{2,\ 153} = 6.69$, $P < 0.001$).

3.2. Impacts on Fallopia japonica

The considerable variation in plant growth recorded in all four traits was not consistently associated with the *A. itadori* strains to which plants were exposed (Fig. 2, Table 2). The only significant effect of strain was detected in the change in plant height, where hybrids (predicted mean [95% confidence intervals]: 352.21% [232.24–472.19]) had least effect in suppressing plant growth (plants had greater percentage changes in height) compared to LTLR (275.23% [155.23–395.22]) and STLR (286.40% [161.65–411.14]) which had similar estimates ('lsmeansLT' estimates: $P = 0.08$ and 0.07, for hybrids vs LTLR and STLR respectively). For plant height, we also found evidence of a differential effect of *A. itadori* strain conditional to SDI: the STLR strain was most effective at reducing maximum height under high SDI values, but least effective under low SDI ($F_{2,44.16} = 4.08$, $P = 0.019$, $N = 54$, 18 plants per strain; Fig. 2C, Table 2). SDI influenced leaf number and above ground weight, with plants having fewer leaves ($F_{1,46.83} = 5.82$, $P = 0.020$) and smaller above ground weight ($F_{1,38.06} = 5.87$, $P = 0.020$) under higher SDI (low humidity).

Out of the four plant growth variables tested, leaf number was the only response variable which was influenced by another predictor besides strain and SDI (other predictors: total eggs, number of adults, week of emergence and initial rhizome weight; see Table A2 for when these predictors were included in our models), where higher rhizome weights at the start of the experiment were associated with more leaves ($F_{1,\ 47.01} = 9.29$, $P = 0.004$; Table 2). None of the variables we tested explained change in rhizome weight (Table 2; Fig. 1A).

4. Discussion

Our study aimed to improve biocontrol of *F. japonica* by exploring the effectiveness of different *A. itadori* strains. We hypothesised that strains which had spent less time in the laboratory (STLR and hybrid strain) would have undergone less selection pressure to perform better under standard laboratory conditions, and therefore would perform better under altered climatic conditions. Previous studies have shown that laboratory rearing may lead to reduced genetic variability compared to wild stocks due to population bottlenecks and selection (Huho et al., 2007; Sgrò and Partridge, 2000), and therefore laboratory stocks tend to become more stress sensitive as selection for stress-related traits is relaxed (Hoffmann and Ross, 2018). Our results did not consistently support this hypothesis suggesting longer time spent in laboratory culture alone does not explain the performance of *A. itadori* as a biocontrol for *F. japonica*.

Table 1
Coefficient estimates for the model predicting *Aphalara itadori* adult survival to adult emergence as a function of total number of *A. itadori* eggs, Saturation Deficiency Index value (SDI), and *A. itadori* strain (LTLR = Long-term laboratory reared; STLR = short-term laboratory reared and Hybrid strain); and adult development as a function of time (in weeks), Saturation Deficiency Index value (SDI), and *A. itadori* strain. We report best parameter estimates (β), their 95% confidence interval (CI), *P*-value, and the number of plants used in each analyses (*N*). The strain reference level (e.g. 'LTLR') is indicated in parentheses. The colon separating variable names indicates interaction terms. Significant variables are highlighted in bold.

Variable	β	Lower 95% CI	Upper 95% CI	*P*-value
Survival ($N = 53$)*				
Intercept (LTLR)	66.05	50.23	81.88	< 0.001
SDI	0.54	−0.28	1.37	0.204
STLR	−2.93	−16.71	10.86	0.679
Hybrid	**−31.71**	**−45.67**	**−17.76**	**< 0.001**
Total eggs	**−0.02**	**−0.03**	**−0.01**	**0.007**
Development ($N = 54$)				
Intercept (LTLR: Low)	78.05	69.42	86.69	< 0.001
STLR	**−20.91**	**−30.29**	**−11.54**	**< 0.001**
Hybrid	**−10.23**	**−19.67**	**−0.78**	**0.035**
High	**−32.64**	**−40.26**	**−25.02**	**< 0.001**
LTLR: Week 1–2	**−56.54**	**−67.23**	**−45.85**	**< 0.001**
LTLR: Week 2–3	**−77.62**	**−88.31**	**−66.93**	**< 0.001**
STLR: Week 1–2	**33.07**	**19.98**	**46.16**	**< 0.001**
STLR: Week 2–3	**29.67**	**16.58**	**42.76**	**< 0.001**
Hybrid: Week 1–2	**14.72**	**1.63**	**27.81**	**0.029**
Hybrid: Week 2–3	**15.96**	**2.86**	**29.05**	**0.018**
High: Week 1–2	**53.17**	**42.48**	**63.86**	**< 0.001**
High: Week 2–3	**44.75**	**34.06**	**55.43**	**< 0.001**
Total eggs	0	−0.01	0.01	1

* One STLR plant had extreme adult A. itadori numbers emerging was removed from analysis.

Among the strains, hybrids had lower survival and developed at a slower rate compared to the LTLR strain. Although the hybrid was created from two genetically different strains (Andersen et al., 2016), differences in the single-nucleotide polymorphisms (SNPs) may not have matched differences in functional gene regions linked to the traits we were assessing. In addition, although there have been studies which have shown improved hybrid fitness, for example in ornamental pear tree *Pyrus calleryana* (Culley and Hardiman, 2009), hybridisation in our study could have led to reduced, rather than improved, fitness. Between-population crosses from Bremgartewald and Spilwald strains of the black timber bark beetle, *Xylosandrus germanus,* were found to be less fit compared to inbred individuals (Peer and Taborsky, 2005). Hybrids from populations of the intertidal copepod species *Tigriopus californicus* also exhibited the negative effect of outbreeding depression, with hybrid fitness initially lower in terms of survivorship and morphology (Hwang et al., 2011). In our study, the hybrid strain was created from the combination of males from the Kyushu strain, which performs best on *F. japonica* compared to other knotweeds, and females from the Hokkaido strain, which oviposit and develop well on giant knotweed, *F. sachalinensis* (Grevstad et al., 2013). It is possible that hybrid breakdown may have occurred whereby the Hokkaido strain's adaptation and preference to living on *F. sachalinensis* was expressed in the hybrids, explaining the low survival to adult emergence observed in the hybrid strain compared to other strains. However, it is important to note that the hybrid was equal to the other two strains observed in terms of reducing the plant growth predictors assessed, and future work assessing more traits would further aid in determining the performance of hybrid strains.

Our study found that *Aphalara itadori* development was slower

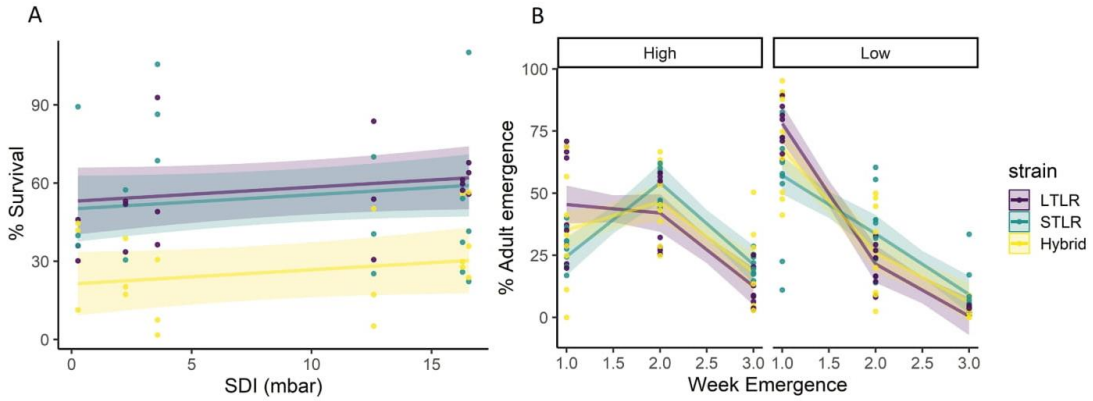

Fig. 1. Relationship between *Aphalara itadori* performance in terms of (A) percentage *A. itadori* survival to adult emergence versus Saturation Deficiency Index (SDI) in treatment cages and (B) *A. itadori* development rate per week. Data points show the observed survival of three *A. itadorii* strains (LTLR = Long-term laboratory reared; STLR = short-term laboratory reared and Hybrid strain) reared on *Fallopia japonica*. Lines show the predicted relationship with SDI from a linear mixed effects model with shaded areas indicating 95% confidence intervals.

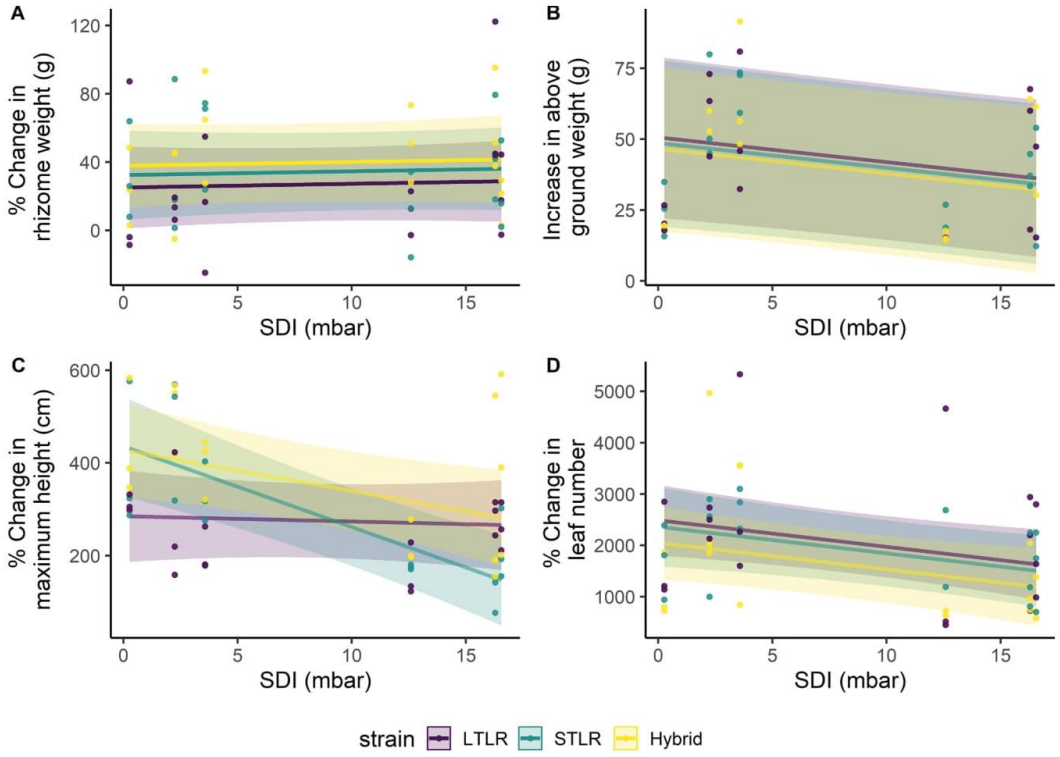

Fig. 2. Relationship between growth of *F. japonica* versus Saturation Deficiency Index (SDI) in treatment cages. Data points show the observed survival to adult emergence of three *Aphalara itadori* strains (LTLR = Long-term laboratory reared; STLR = short-term laboratory reared and Hybrid strain) reared on *Fallopia japonica*. Plant growth was measured using (A) rhizome weight, (B) above ground weight, (C) maximum height, and (D) leaf number. Lines show the predicted relationship with SDI from a linear mixed effects model with shaded areas showing 95% confidence intervals.

under high SDI, which has also been found for other psyllid species (see Hodkinson, 2009). Slower development could explain why plants exposed to *A. itadori* under stressful low humidity levels (high SDI) had lower growth in above-ground weight, height and number of leaves, compared to plants under high humidity levels. The more damaging nymphal stage of *A. itadori* is extended under slower development (Djeddour and Shaw, 2010) and therefore the per capita impact of individuals is likely to increase, potentially making them more effective

Table 2
Summary of models predicting percentage change in *Fallopia japonica* factors as a function of Saturation Deficiency Index (SDI), *Aphalara itadori* strain (LTLR = Long-term laboratory reared; STLR = short-term laboratory reared and Hybrid strain), the total number of *A. itadori* adults produced at the end of the experiment, and the initial rhizome weight. We report best parameter estimates (β), their 95% confidence interval (CI), *P*-value, and the number of plants used in each analyses (*N*). The strain reference level (e.g. 'LTLR') is indicated in parentheses. The colon separating variable names indicates interaction terms. Significant variables are highlighted in bold.

Variable	β	Lower 95% CI	Upper 95% CI	*P*-value
Rhizome weight (N = 54)				
Intercept (LTLR)	27.32	−3.89	58.53	0.109
SDI	0.22	−1.03	1.47	0.729
STLR	7.29	−13.86	28.44	0.503
Hybrid	12.77	−12.45	37.98	0.326
Number of adults	−0.01	−0.06	0.04	0.770
Above ground weight (N = 46)*				
Intercept (LTLR)	57.97	27.22	88.73	0.020
SDI	**−0.87**	**−1.58**	**−0.17**	**0.020**
STLR	−2.01	−13.79	9.77	0.740
Hybrid	−4.00	−18.61	10.61	0.594
Number of adults	−0.02	−0.05	0.00	0.106
Initial Rhizome weight	−0.00	−0.13	0.12	0.940
Maximum height (N = 54)				
Intercept (LTLR)	340.49	204.08	476.90	< 0.001
SDI	−1.14	−8.30	6.01	0.755
STLR	**150.85**	**42.17**	**259.54**	**0.009**
Hybrid	**142.80**	**23.88**	**261.72**	**0.023**
SDI: STLR	**−16.27**	**−26.10**	**−6.45**	**0.002**
SDI: Hybrid	−7.67	−17.33	2.00	0.127
Number of adults	−0.15	−0.32	0.02	0.081
Initial Rhizome weight	−0.12	−0.94	0.69	0.769
Leaf number (N = 54)				
Intercept (LTLR)	1622.59	493.61	2751.57	0.009
SDI	**−51.75**	**−93.79**	**−9.71**	**0.020**
STLR	−127.08	−833.21	579.04	0.726
Hybrid	−438.50	−1272.10	395.10	0.308
Number of adults	−0.26	−1.88	1.36	0.754
Initial Rhizome weight	**12.48**	**4.45**	**20.50**	**0.004**

* Eight *F. japonica* (three LTLR, one STLR and four Hybrid) had weights missing and were removed from analysis.

biocontrol agents under high SDI conditions. Indeed, we found that the STLR strain developed slowest and had a greater impact on plant height under high SDI. However, this benefit could be offset by there being fewer generations per season, something that will need to be confirmed in future studies.

The finding that *A. itadori* survival was not influenced by SDI contrasts with other studies on other psyllid species that have shown that high SDI leads to lower survival (Hall and Hentz, 2001; Hodkinson, 2009; McFarland and Hoy, 2001). These differences may reflect variation among species, but it is also possible that our hotter, drier conditions were not sufficiently stressful to induce mortality. The experiments were done within a greenhouse where conditions limited our ability to strictly control temperature and humidity.

Due to space limitations in the quarantine area we could not assess how environmental conditions affected plants without *A. itadori*. However, the reduced above-ground biomass and number of leaves observed in plants under high SDI could reflect more stressful conditions for the plants, especially as all plants were regularly watered, so only ambient humidity changed. If plants by themselves were not affected by the more stressful ambient conditions in the experiment, this suggests that *A. itadori* could be even more damaging when plants are exposed to high stress conditions in the field.

Notably, we found no effects of strain or SDI on rhizome weight. This could be because both insects and ambient humidity do not directly affect rhizomes, and nutrient availability in the soil was sufficient to avoid rhizome depletion associated to above ground growth. Since *F. japonica* is mainly spread by pieces of rhizome this highlights the challenge in developing an effective biological control to reduce the spread of this invasive plant, however further generations of psyllid development on the same plant may lead to rhizome depletion in the longer term.

Overall, our results do not support a beneficial role of intraspecific variation in the biocontrol effectiveness of *A. itadori* for the strains considered. Genetic work would be necessary to reveal if this is due to genetic variability being different from our hypothesis (lower in LTLR and highest in hybrids). Additional work under laboratory and field conditions would also be necessary to test a wider range of climate conditions (as responses are likely to be non-linear), to evaluate cross-generational changes including hybrid fitness after more generations, to assess a broader genetic range of *A. itadori* populations and to take into account additional factors such as predator avoidance and overwintering performance. Effectively controlling *F. japonica*, both above and below ground, is still the challenge ahead.

CRediT authorship contribution statement

Chanida Fung: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Pablo González-Moreno:** Conceptualization, Methodology, Funding acquisition, Supervision, Writing - review & editing. **Corin Pratt:** Conceptualization, Methodology, Investigation, Writing - review & editing. **Tom H. Oliver:** Formal analysis, Funding acquisition, Writing - review & editing. **Robert S. Bourchier:** Conceptualization, Methodology, Funding acquisition, Writing - review & editing. **Manuela González-Suárez:** Conceptualization, Methodology, Formal analysis, Funding acquisition, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A

Table A1
Alternative methods of calculating Saturation Deficiency Index value (SDI) adapted from (Green and Catling, 1971). Maximum temperatures were the maximum temperatures across the whole experiment.

	SDI methods
1	Mean of 3 highest daily maximum temperatures with the mean of the three vapour pressures coinciding with the 3 highest maximum temperatures
2	Mean of the 3 SDI values (millibars) coinciding with the 3 highest maximum temperatures

Table A2
All models tested to analyse the effects of strain and Saturation Deficiency Index value (SDI) on *Aphalara itadori* performance (survival to adult emergence and development) and *A. itadori* impact on *Fallopia japonica* growth (rhizome weight, above ground weight, maximum height and leaf number. * indicates tested interactions and models used are highlighted in bold.

Model	Fixed predictors	Random Factors
A.itadori performance		
Survival		
S1	**Strain + SDI + total eggs**	**Batch + observer**
S2	Strain*SDI + total eggs	Batch + observer
Development		
D1	Strain + SDI + week emerge + total eggs	Batch + observer
D2	Strain*SDI + week emerge + total eggs	Batch + observer
D3	**Week emerge*(strain + SDI) + total eggs**	**Batch + observer**
D4	strain*(SDI + week emerge) + total eggs	Batch + observer
Impact on *F. japonica*		
Rhizome weight		
R1	**Strain + SDI + total number of adults**	**Batch**
R2	Strain*SDI + total number of adults	Batch
Above ground weight		
A1	**Strain + SDI + total number of adults + before rhizome weight**	**Batch**
A2	Strain*SDI + total number of adults + before rhizome weight	Batch
Maximum height		
H1	Strain + SDI + total number of adults + before rhizome weight	Batch
H2	**Strain*SDI + total number of adults + before rhizome weight**	**Batch**
Leaf number		
L1	**Strain + SDI + total number of adults + before rhizome weight**	**Batch**
L2	Strain*SDI + total number of adults + before rhizome weight	Batch

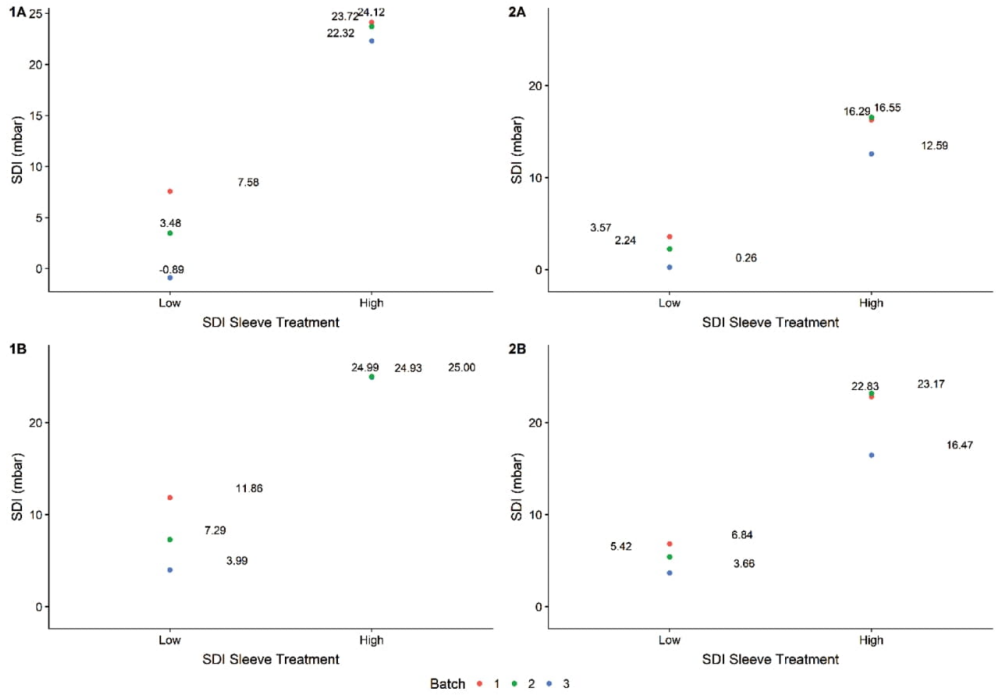

Fig. A1. Four methods chosen for calculating Saturation Deficiency Index value (SDI) adapted from (Green and Catling, 1971). Points are the calculated SDI values of dataloggers for each experimental batch. Each datalogger was placed in one sleeve within a treatment cage. (1A) SDI was firstly calculated per day by taking the mean of the top three temperature values and its corresponding relative humidity values (RH). The final SDI value assigned to the batch was the average SDI for the whole experiment. (1B) SDI was firstly calculated per day by taking the mean of the top three temperature values and corresponding RH values. The final SDI value was than assigned by calculating the mean of the top three SDI values for the whole experiment. (2A) SDI values were calculated for each reading (30 min) and the mean of the highest three SDI values was obtained. The final SDI value assigned to the batch was the average SDI for the whole experiment. (2B) SDI values were calculated for each reading (30 min) and the mean of the highest three SDI values was obtained. The final SDI value assigned by calculating the mean of the top three SDI values for the whole experiment.

Fig. A2. Experimental *Fallopia japonica* plants. a) For the experiment, plants were placed in a 16.5 cm diameter saucer within a humidity cage with capillary matting. They were irrigated twice a week manually; b) after egg counts, plants were covered in 1 m long insect sleeves, tied with elastic bands and supported by bamboo halos to avoid *Aphalara itadori* escaping.

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