The biology and ecology of *Bruchus rufimanus* (bean seed beetle)

Rebecca Louise Ward

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School of Natural and Environmental Sciences

Newcastle University

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Declaration

I declare that this work has not already been accepted for any degree and is not currently submitted in candidature for any degree other than the degree of Doctor of Philosophy of Newcastle University. I declare that this is my own work and that the use of all material from other sources has been properly and fully acknowledged.

Signed:...................................... Date:..............................

Name: Rebecca Louise Ward

Abstract

The bean seed beetle, *Bruchus rufimanus* (Boheman), is an economically important pest of *Vicia faba* bean crops throughout Europe and has become established as a serious pest in the UK since 1990. Damage reduces the value of the dried *V. faba* crop and the presence of larvae or unemerged adults in the beans results in crop rejection for all the main quality markets. The life cycle is not completed in fresh harvested broad beans, but immature beans show damage and contain the pest larvae. Affected broad beans crops are rejected, resulting in a total loss to the grower.

The main objectives of the research were as follows: to improve knowledge of factors influencing the emergence of insects from their over-winter habitats and factors leading to the termination of diapause; to improve knowledge of conditions that influence oviposition and damage, such as feeding opportunities, temperature, photoperiod and pollen type needed to stimulate oviposition; to improve knowledge of factors influencing the selection of or preference for over-wintering habitats; and to investigate the distribution of the pest throughout the UK. The influences of host plant sowing date, cultivar and density were investigated.

The research provided evidence for the influence of temperature on damage caused by *B. rufimanus* and provided guidance for the distribution of the pest across the UK, allowing growers to plan insecticide applications according to regional pest pressure. It has been possible to link these findings with data supporting improved cultural methods of reducing the impact of the pest, particularly the timing of sowing.

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Chapter 1: Introduction

Bruchus rufimanus Boheman is a member of the order Coleoptera, family Chrysomelidae, sub-family Bruchinae. Common names include the bean seed beetle, broad bean weevil and in the UK, the bruchid beetle. It is one of several species of the genus Bruchus that have been recorded in the UK and include *B. loti* (Paykull), *B. lentis* (Frohlich), *B. atomarius* (Linnaeus), *B. rufipes* (Herbst), *B. affinis* (Frohlich) and *B. pisorum* (Linnaeus).

It is an economically important pest of *Vicia faba* Linnaeus throughout Europe and has become established as a serious pest in the UK since the early 1990's. The beetle is univoltine and adults hibernate during winter in sheltered habitats, emerging in April and May to feed in flowering *V. faba* crops (Tran *et al*., 1993). Following an obligatory period of feeding on the pollen on *Vicia* or *Lathyrus* species (Delobel and Delobel, 2006), during which reproductive diapause terminates, female *B. rufimanus* oviposit onto developing pods, predominantly at the base of the plant where flowering starts (Ward, 1999).

Hatching larvae bore through the base of the egg and the *V. faba* pod walls, and larvae feed in the developing seed. When fully grown, larvae pupate and adult *B. rufimanus* emerge at crop senescence, leaving a round hole, approximately two to three millimetres in diameter in the *V. faba* grain. Damage to grain quality affects the end use, and major existing markets for *V. faba* grown in the UK include those for UK animal feed, seed and for export for human consumption, with niche markets for pigeon feed, aquaculture and developing snack products (Redman, 2015). *V. faba* is exported from the UK to Africa and the Middle East, mainly Egypt, for use in products such as falafel and Ful Medames (Redman, 2015). There is considerable variation in the quantity of *V. faba* exported for human consumption each year depending on the quality of the beans, and grain merchants exercise strict quality requirements for freedom from staining and pest damage. For exported grain intended for human consumption, damage caused by *B. rufimanus* must not exceed 2% in *V. faba* produced in the UK (P. Brown, 2015, Personal Communication). For *V. faba* grain intended for animal feed, the visual quality of grains is not considered important, as most is used as a milled component in compound feed. It is not necessary to control *B. rufimanus* damage in this case. Opinion varies regarding the damage caused to germination capacity in *V. faba* grain by *B. rufimanus*, although it is likely that smaller seeded cultivars and grain that is stored for longer periods may be more susceptible to germination losses. Grain containing drill holes caused by *B. rufimanus* may also be more susceptible to fungal pathogen infections (Jones and Jones, 1964). While there is no recent data to support this, Keneni *et al.* (2011) suggested that legumes damaged by a range of pests, including *B. rufimanus,* may incur additional losses in germination capacity, grain spoilage and bad smell.

V. faba provides a number of benefits for human and animal nutrition, such as high protein and essential amino acid content (Duc, 1997; Crepon *et al.*, 2010), and to the environment. Biological nitrogen fixation is considered to be one of the most important benefits provided by cultivation of *V. faba* (Kopke and Nemecek, 2010) and UK production area in 2015 increased by 50%, to approximately 213,000 hectares (EUROSTAT, 2016), following measures to allow nitrogen fixing crops to be eligible for grant payments within the reformed Common Agricultural Policy (European Commission, 2016). Other benefits include the reduction of energy use and climate impact, spatial and temporal diversification and the provision of pollinator resources (Kopke and Nemecek, 2010; Redman, 2015), and *V. faba* should be considered as a potentially profitable break crop in agricultural rotations.

Management of *B. rufimanus* has proved difficult and the choice of insecticides approved in *V. faba* in the UK is currently limited to pyrethroids (FERA, 2016), which are applied during flowering and early pod formation. The impacts of pyrethroid insecticides on bees are well known and include effects on foraging behaviour, motor function, grooming and wing fanning behaviour, as well as direct toxic contact effects (Vandame and Belzunces, 1998; Gill and Raine, 2014; Sanchez-Bayo and Goka, 2014; Oliver *et al*., 2015).

The principal objective of the research was to improve management of *B. rufimanus* in *V. faba* and in particular to:

- Investigate the distribution of *B. rufimanus* throughout the UK in order to inform *V. faba* growers of regional risk;
- Improve knowledge of the factors influencing the emergence of *B. rufimanus* from over-winter habitats and factors leading to the termination of reproductive diapause;
- Provide a robust strategy for field control, including timing of insecticides to optimise efficacy whilst reducing impact on beneficial organisms;

- Improve knowledge of the conditions required for oviposition to commence, such as feeding opportunities, temperature, photoperiod and pollen type needed to stimulate oviposition;
- Provide alternative strategies to manage *B. rufimanus*, including the use of cultural and rotational techniques to reduce insecticide inputs and optimise financial return to growers;
- Improve knowledge of the factors influencing the selection of or preference for over-wintering habitats;

Chapter 2: Literature review

2.1 Faba bean (*Vicia faba* Linnaeus)

The faba bean (*Vicia faba* Linnaeus) is a member of the family Fabaceae (Leguminosae), subfamily Papilionoideae, tribe Fabae. It is known to have been cultivated during the early Neolithic period in the Near East and possibly the Mediterranean region (Cubero, 1974; Duc, 1997), and is now widely cultivated globally. Cubero (1974) described four groups of *V. faba*, *equina*, *major*, *minor* and *paucijuga*, and proposed that *equina* and *major* evolved from a primitive group classified archaeologically as *minor*, and that the current *minor* and *paucijuga* are relicts. Cubero (1974) considered that the groups were likely to have been dispersed indirectly through knowledge of cultivation of wild types, rather than direct seed transmission, although *V. faba minor* is likely to have been more dependent on transmission of seed by humans. The origin of *V. faba* is still unknown as no wild progenitor has been found (Duc, 1997). Common names for *V. faba* include field bean, tick bean or pigeon bean (*minor*), broad bean or Windsor bean (*major*) and the horse bean (*equina*). For the purposes of this document the common names field bean or broad bean will be used where the scientific name is substituted.

V. faba is an annual plant which may be planted in the autumn or spring and in the UK there has been an increase in the production of spring-sown field beans since 1987 (Ward, 1999). The root system consists of a tap root and secondary roots and bears nodules containing *Rhizobium leguminosarum* bv. *viciae* (Duc, 1997), nitrogen-fixing bacteria. Plant growth is indeterminate, leading to extended flowering and a variable number of flowering nodes per plant (Duc, 1997) (Appendix A). In combination with continued production of autumn sown field beans that flower earlier than those that are spring-sown, an extended flowering period is experienced in the UK in most years.

V. faba provides a number of important benefits to human and animal nutrition and the environment. Protein content can range between 27 and 34% of seed dry matter (Duc, 1997), made up of the amino acids lysine, methionine, cysteine and tryptophan (Crepon *et al.,* 2010), which are important for human and animal nutrition. *V. faba* and other pulses form a critical part of the human diet for this reason (FAO, 2016). *V. faba* also contains constituents that exert anti-nutritional effects for both animals and humans, and tannins, vicine and convicine have been shown to adversely affect animal nutrition (Jansman *et al.,* 1993; Vilarino *et al.,* 2009; Crepon *et al.,* 2010). In human nutrition, the components vicine and convicine are toxic to those with the genetic disorder favism, which causes acute haemolytic anaemia (Davies, 1961). In addition to the anti-nutritional effects of legumes, there is evidence of phytotoxicity to specific insect colonisers (Holloway, 1986; Gbaye *et al.,* 2011).

Kopke and Nemecek (2010) carried out a substantial review of evidence for the ecological services provided by *V. faba* cropping, listing biological nitrogen fixation by symbiosis with *R. leguminosarum* bacteria as the principal service. This contributes to protein content of *V. faba* and has the potential to reduce the mineral nitrogen fertiliser requirement in the crop rotation, although variable levels of biological nitrogen fixation are recorded for *V. faba* (Sprent and Bradford, 1977; Walley *et al.*, 2007). The production of mineral nitrogen fertiliser has significant impact on fossil energy use, contributing about 50% of the total energy input per hectare in intensive arable areas (Kopke and Nemecek, 2010), and thus reduction of use has the potential to provide significant energy saving and climate impact. Inclusion of *V. faba* in rotations also offers growers the opportunity for both spatial and temporal diversification in crop rotations, with consequent benefits for the environment, in particular the benefits to pollinators. *V. faba* is visited by honeybees (*Apis mellifera* Linnaeus) in most countries, *Bombus* sp. in temperate regions, particularly *Bombus terrestris* (Linnaeus) in the UK, and solitary bees (*Eucera nigriscens* Perez and *Anthophora* sp.) in a variety of climates (Duc, 1997). The *V. faba* flower has a nectary at the base of the pistil, flower volatiles attractive to bees, and pollen at the front of the flower (Stoddard, 2017). Venation of keel petals and dark spots on the wing petals help to guide bee landing. Breeze *et al.* (2011) studied pollination services provided by honeybees in the UK, acknowledging the role that bumble bees play and concluding that wild pollinators play a greater role in providing pollination services to crops than previously thought. The authors calculated that the overall value of all UK pollinated crops in 2011 was £1,057.8 million, with an area representing 20% of all UK cropped land. Cunningham and Le Feuvre (2013) calculated that the provision of honeybee hives led to mean yield increases of 17% in *V. faba* crops. The increasing importance of honey bees may in part be due to the overall decline in diversity and abundance of wild pollinators such as bumblebees, solitary bees, hoverflies, wasps and butterflies (Bailes *et al.,* 2015). This decline has been attributed to land use change and the reduction of the area available for life-cycle completion, particularly the cultivation of crop species with floral resources that are available for short periods only. *V. faba* provides abundant floral resources for pollinating insects (Nayak *et al.,* 2015) and recent change to Common Agricultural Policy in the EU, allowing growers to include peas and beans as part of their ecological focus area under the updated greening obligations (European Commission, 2016), led to a significant increase in the area of *V. faba* cultivation in the UK, with a consequent increase in floral resource availability for pollinators. Nayak *et al.* (2015) found that open pollination of *V. faba* crops compared with autonomous self-pollination and wind and self-pollination provided significant increases in pod set per flower, number of beans per pod, individual bean weight and total bean weight per square metre. There is considerable variation in the level of autofertility of different cultivars of *V. faba* (Marcellos and Perryman, 1990) and much discussion regarding the contribution of insect pollination to yields (Stoddard, 1986; Suso *et al*., 1996), but it is generally considered that pollination from bees and beneficial insects provides between 30 and 60% of the pollination requirement of *V. faba* (Delaplane and Mayer, 2000; Cunningham and Le Feuvre, 2013) and in some cases more. The role of insect pollination in *V. faba* is considered here in relation to current control and management of *B. rufimanus* which, in most countries, is undertaken using broad spectrum pyrethroid or neonicotinoid insecticide applications.

Global production of *V. faba* was estimated at 3.32 million hectares in 2014 (FAOSTAT, 2016), although the published figure for the UK was 21,400 hectares (unofficial), a great underestimation compared to the EUROSTAT figure for 2014 (107,000 hectares) (EUROSTAT, 2016) which accurately represented UK production (figures for other European countries appear to be correct within FAOSTAT compared to EUROSTAT). The UK was therefore the fifth largest individual producing country of *V. faba* in the world in 2014 (Figure 2.1) and area increased to 170,000 hectares in 2016 (provisional) (EUROSTAT, 2016).

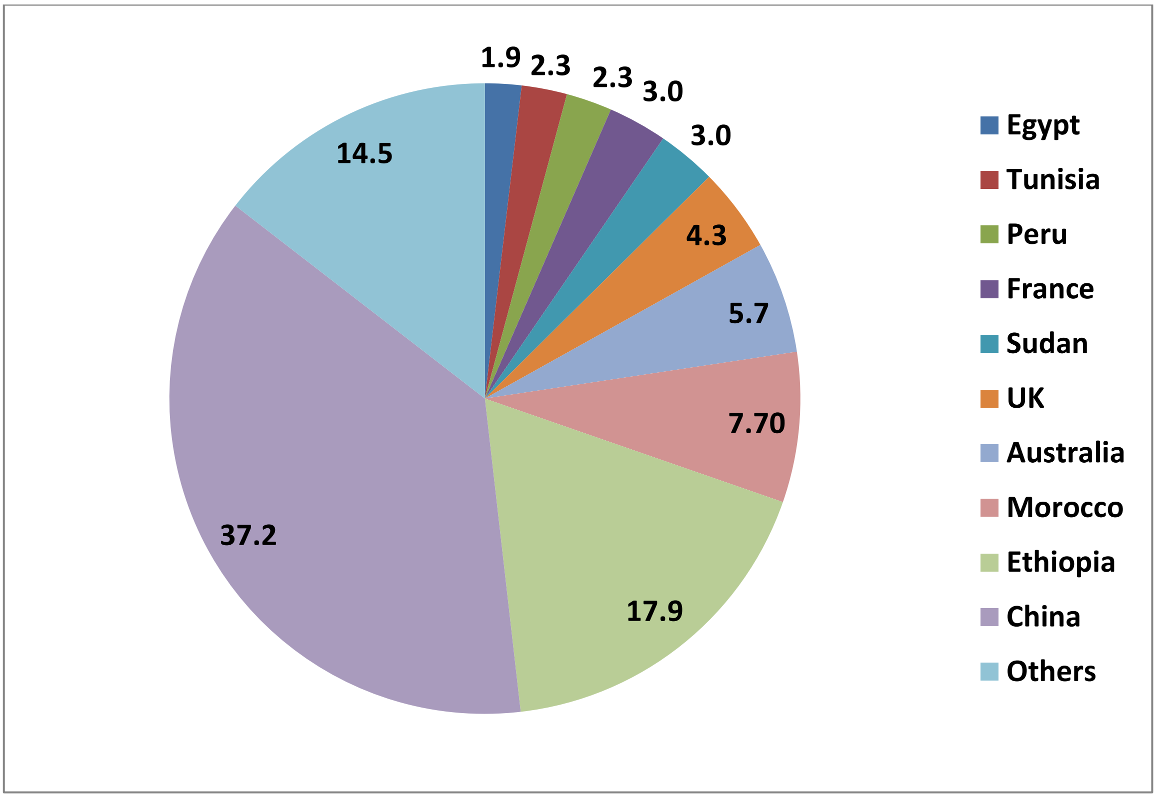


Figure 2.1: Area of production (hectares) of *V. faba* in the 64 producing countries of the world represented as percentage of global production (from FAOSTAT and EUROSTAT data for 2014).

V. faba has traditionally been grown in the UK for horse feed and following a peak cropping area of 224,000 hectares in 1873 (Knott, 1997) the area declined until 1978 when European Community subsidies were introduced to encourage home grown protein production for animal feed. By 1993 the area reached 163,000 hectares. Knott (1997) described low, unstable yields of *V. faba* up until 1976 due to drought, disease and harsh winter conditions, and subsequent improvements in plant breeding for characters such as standing ability, harvestability, quality, maturity and disease resistance. Suso *et al.* (1996) and Duc (1997) also described yield instability in *V. faba*. Although the five year average UK yield increased from 2.66 tons per hectare in 1976 to 3.58 tons per hectare in 1986, Knott (1997) showed that it reached a plateau from 1986 until 1993 of between 3.2 and 3.4 tons per hectare. Despite breeding improvements, yield has remained relatively static in recent years, although improvements have been recorded in Recommended List trials in 2014 and 2015 (PGRO, 2016).

Major existing markets for *V. faba* grown in the UK include those for UK animal feed and for export for human consumption, with niche markets for pigeon feed, aquaculture and developing snack products. Field beans are not consumed in large quantities by humans in the UK but exported to the Middle East, mainly Egypt (Redman, 2015), for use in products such as falafel and Ful Medames. There is considerable variation in the quantity of *V. faba* exported to the Middle East for human consumption and this is dependent on the quality of the beans. Redman (2015) estimated that between 60 and 70% of UK production was exported for the human consumption market by 2014, with the remainder used in UK animal feed rations. This represents a major change in UK market dynamics since 1997, when export of field beans for human consumption was 15,000 tons, or around 4% (Ward, 1999), and also presents a great challenge to growers to produce high quality field beans. Traders exercise strict quality requirements for freedom from staining and pest damage, and damage caused by *B. rufimanus* must not exceed 3% damaged seeds (France) to reach human consumption quality for export (INRA, 2016) or 2% in crops produced in the UK (P. Brown, 2015, Personal Communication).

2.2 Bean seed beetle (*Bruchus rufimanus* Boheman)

2.2.1 *Taxonomy and history*

Bruchus rufimanus Boheman, 1833 is a member of the order Coleoptera, family Chrysomelidae, sub-family Bruchinae. It was previously classed as family Bruchidae until recent reclassification following partial reconstruction of the phylogeny of European seed beetles (Kergoat *et al*., 2004). It has the synonyms *Bruchus affinis* sensu auct. Brit. not Froelich, 1799 and *Bruchus velutinus* Mulsant and Rey, 1858 (Natural History Museum, 2016). Common names include the bean seed beetle, broad bean weevil and more locally, the bruchid beetle. The term bruchid beetle is used commonly in the UK, as *B. rufimanus* is the only Bruchinae species that affects commercial cropping at present. The sub-family Bruchinae consists of about 1,300 species of seed or bean weevils (Johnson *et al.,* 2004) and is split into two groups (Southgate, 1979; Pajni, 1987). In one group, to which *B. rufimanus* belongs, insects are univoltine and adult females must lay eggs on the surface of the pods in the field. The larvae develop in the seeds and the adults either emerge at harvest, moving to sheltered winter sites, or they remain in the seed until the following year, doing no further damage during storage. In the second group the adult females lay eggs onto pods or seeds and breeding may continue during storage. Storage bruchids have shorter life-cycles and reproduce more rapidly, allowing potential for multiple generations throughout the year.

B. rufimanus is present globally where *V. faba* is cultivated for dry harvest and has since 2000 been recorded as an invasive species in Western Australia (CSIRO, 2004; PaDIL,

2011) for the first time. Borowiec (1987) described the distribution of *Bruchus* genus as the Palearctic region, including the Far East, northern India, tropical Africa, South Asia and North America. It has long been present in the UK, Europe, Asia, North America and Africa and has been recorded in the UK from archaeological samples collected in Lincoln originating from the Saxon or early medieval periods (Carrott *et al*., 1995). The origin of *B. rufimanus* appears to be unknown and it cannot be assumed that its evolution followed that of *V. faba*. Ehrlich and Raven (1964) proposed that co-evolution exists between phytophagous insects and their host plants, based on Lepidoptera-host-plant associations, and that both plant and insect species stimulate evolution in the other, the plants by chemical responses to insect attack and the insects by adaptation to the chemical responses. This is not supported for *Bruchus* (Jermy and Szentesi, 2003), following a long-term Hungarian study that concluded that it was more likely that legumes evolved into the extant species and that bruchids adapted to them without affecting the macroevolution of legumes. Kergoat *et al.* (2004) indicated the potential for rapid diversification among bruchids while acknowledging the limitations of current data in elucidating the evolution of bruchids. Delobel and Delobel (2006) supported this finding in their study of dietary specialisation in European Bruchinae, discussing the strong relationship that seed beetles have with their host plants and concluding that *B. rufimanus* feeds on plants of both *Lathyrus* sp. and *Vicia* sp., indicating a single shift to Vicieae followed by adaptive radiation on genera *Vicia* and *Lathyrus*.

2.2.2 *Description and life-cycle*

Borowiec (1987) characterised the genus *Bruchus*, distinguishing it from all other genera of Bruchinae using specific characters. These are described as being pronotum with lateral denticle, the structure of the mid-tibia in male, and the unique structure of median lobe and parameres. Kingsolver (2004) provided a detailed description of *B. rufimanus*, using a comparison with *Bruchus pisorum* Linnaeus*,* a species affecting *Pisum sativum* Linnaeus (pea), to distinguish the species. *B. rufimanus* and *B. pisorum* are similar in size and general appearance but the lateral denticle of the meta-femur and the black subapical spots on the pygidium are smaller in *B. rufimanus*. He cites the swollen mesofemur and sinuate mesotibia of male *B. rufimanus* as being useful distinguishing characters. *B. rufimanus* adults are dark brown to black in colour, 3.1 to 4.4 mm long, with white or grey markings on the elytra caused by the presence of white hairs (Ward, 1999; Kingsolver, 2004). The four basal antennal segments and fore-legs are red (Hoffmann *et al.*, 1962). The head and pronotum are yellow to brown, and the lateral pronotal margin and discal spots are white. The pygidium is exposed by shortened elytra (Hoffmann *et al.*, 1962) and is pale in colour, the apex truncate in males and evenly curved in females. Antennae are angled at the fifth segment and the terminal segment is ovate. The species has four larval instar stages (Hamani and Medjdoub-Bensaad, 2015) and the final instar reaches a length of 4.0 to 5.5 mm (Pfaffenberger, 1977). The cuticle of the final instar is white to yellow, and the larva has a distinct light brown coloured head and segmented antennae located at the base of the mandible. The clypeolabrum is lightly pigmented.

B. rufimanus is univoltine, a characteristic shared by many bruchids that cause damage principally during growth of the plant. Bruchids that cause damage only during grain storage may be multivoltine (Pajni, 1987). Diapause takes place either in winter habitats, in particular standing trees and well-established hedgerows, or inside *V. faba* grains during storage. Conditions required for reproductive diapause are described more fully in 2.2.5. Adults emerge from overwintering sites and enter host crops to feed on pollen for several weeks, which females must do in order to terminate reproductive diapause. Adults fly freely, described as travelling up to two kilometres to find pollen sources by Hoffman *et al.* (1962), although Southgate (1979) makes reference to the lack of existing information regarding flight patterns of Bruchinae. There appears to be no more recent study of flight patterns or distance for *B. rufimanus*. Mating occurs, and eggs are laid on the pods of *V. faba*. In this respect *B. rufimanus* may be described as being in oviposition Guild A (Johnson and Romero, 2004), ovipositing only on fruits while on the plant. The eggs are laid singly on pods and protected by a gelatinous substance exuded at oviposition to attach the egg firmly to the pod (Hoffmann *et al.,* 1962; Southgate, 1979). Placement onto the pod is irregular, eggs are laid individually, and position is not related to pod characteristics or the position of the seeds inside the pod (Hoffmann *et al.,* 1962; Ward, 1999). It is, however, related to the position of podded plant nodes and eggs are laid primarily on the lower nodes of plants that are present when oviposition begins. The period of peak oviposition occurs in the initial two weeks of oviposition but may last one to two months depending on region and climate (Hamani and Medjdoub-Bensaad, 2015). The larva exits the base of the egg after two days and bores through the pod wall and into the seed. Larvae do not always enter the seed directly downwards and often leave markings as they move across and through the seed coat. There is no point of exposure of the larva on the pod surface (Hoffmann *et al.*, 1962). Larvae develop in the seeds for between four and five months (Hamani and Medjdoub-

Bensaad, 2015), following which they either emerge as adults at harvest moving to winter habitats whereupon diapause begins, or remain within the seed during diapause.

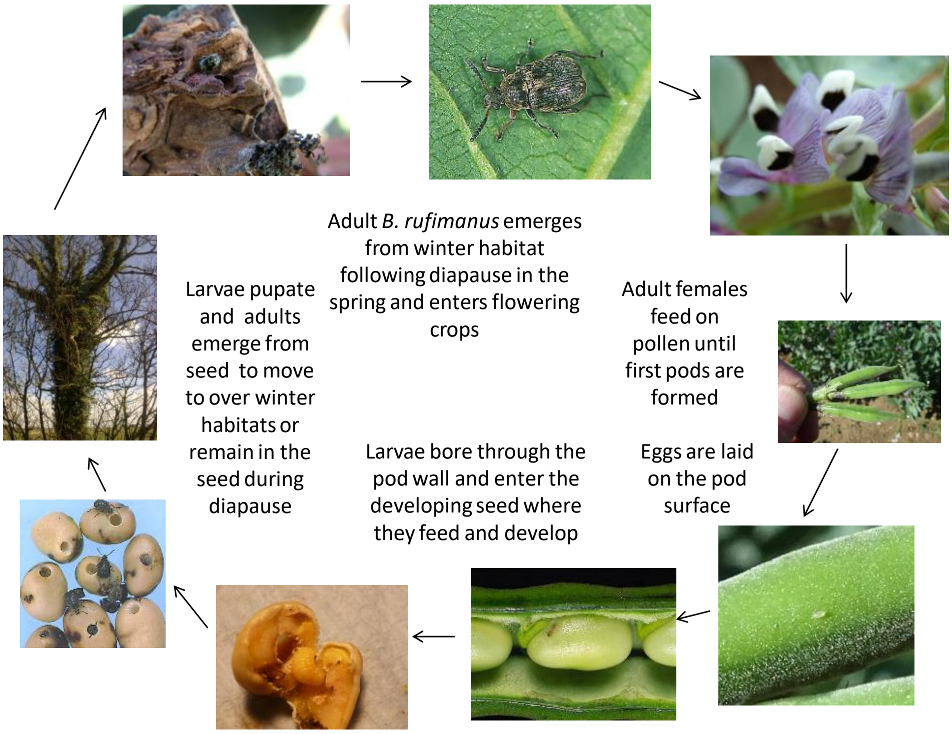


Figure 2.2: Key stages in the life-cycle of *B. rufimanus*. Image of adult *B. rufimanus* courtesy of Nigel Cattlin, all other images produced by R. Ward and property of the Processors and Growers Research organisation.

2.2.3 *Parasitism of B. rufimanus*

Parasitism by Hymenoptera causes some mortality of *B. rufimanus* larvae (Ahmed, 1996). De Luca (1965) listed eight Braconidae, one Eurytomidae, five Pteromalidae and one Trichogramma species that have been found to parasitise *B. rufimanus* globally. *Triaspis luteipes* Thomson and *Dinarmus laticeps* Ashmead (a synonym of *Dinarmus basalis* (Rondani)) were the only two from de Luca (1965) that were recorded as parasites of *B. rufimanus* in the UK. A species found in *V. faba* seeds in Cambridgeshire, UK in 1998 was identified as *T. luteipes*, from the Braconidae family (M. Shaw, 1999, Personal Communication). *Triaspis* species always attack the host early in its life, either while it is a young larva, or possibly at embryo stage in the egg, thus, in the field in the case of *T. luteipes* parasitizing *B. rufimanus*. *Triaspis* is always solitary in the sense that one develops per host, and they mostly attack Bruchinae or Curculionidae. They are internal parasites of the host larva and the host continues to grow after colonisation, most likely being killed as a pre-pupa. The parasitoid larva exits following death of the host and feeds externally before making a cocoon inside the host's pupation chamber (M. Shaw, 1999, Personal Communication). Quantification of the level of parasitisation of *B. rufimanus* by Hymenoptera is not well reported, although Ahmed (1996) reports 30.1% parasitisation by *Anisopteromalus calandrae* Howard (Hymenoptera: Pteromalidae) in laboratory studies. Hymenoptera parasites are not considered to be effective control agents for bruchids (National Research Council, US, 1978) and in the UK *B. rufimanus* populations are not suppressed to commercially acceptable levels by Hymenoptera parasites, despite high numbers being present in seeds in some years (Ward, 1999). Damage caused by parasitic Hymenoptera may also lead to rejection of *V. faba* grain for higher quality markets.

2.2.4 *Symptoms of seed infestation and commercial impact*

Adult *B. rufimanus* leaves a round exit hole as it emerges from the *V. faba* seed and this affects the quality for human consumption, as well as the germination capacity of the seed. At low levels of infestation germination losses may not be significant in larger seeded cultivars, although damaged beans may be more susceptible to moulds (Jones and Jones, 1964; Ward, 1999). Southgate (1979) describes variation in germination losses for different legume species affected by Bruchinae, suggesting that lightly infested seeds have a greater chance of survival, with the size of seed and portion remaining following larval feeding being important determinants of germination capacity. Due to the movement of the *B. rufimanus* larva across and through the testa as it enters the seed, brown marks are present on the surface both at the immature stage harvest for broad beans (*V. faba major*) and at the desiccated (dry) stage for field beans (*V. faba minor*). At the immature stage in broad beans larvae are present in the seeds and at the dry stage adults and underdeveloped larvae may be present. Where adults have not emerged from seed there is frequently a translucent circle in the testa of the seed which protects the adult from the external environment. Damage is caused to the seed of *V. faba* by the presence and activity of parasitic Hymenoptera, which leave small holes in the surface of the seed and unacceptable blemishing.

The symptoms described are used to classify seeds, particularly for field beans traded as dry grains, for their suitability for different end-uses. Data provided by Frontier Agriculture for the years 2006 to 2014 showed that due to the strict requirements in the UK for export quality human consumption *V. faba* grain (2% *B. rufimanus* damage), between 10 and 55% of annual production has reached this standard since 2006. Thus, in some years 90% of UK production does not reach the quality required for export for human consumption. The cost of insecticides to control *B. rufimanus* is approximately £8 per hectare for two applications of a pyrethroid insecticide, or £2 per ton *V. faba* produced (C. Allen, 2017, Personal Communication), based on average yield at 4 tons per hectare (EUROSTAT, 2016). A production premium exceeding the value of beans used in animal feed rations is paid to growers that produce beans of the required standard for human consumption. In 2015 this was £25 per ton and in 2014 £30 per ton. For 2014, as an example, the estimated loss to *V. faba* growers in premiums for human consumption, based on production of 448,000 tons (EUROSTAT, 2016) and 59.1% of production being below the required quality standard (Frontier Agriculture Ltd., 2015, Personal Communication), was £7.94 million. Using Redman's (2015) estimate that 60 to 70% of *V. faba* was exported for human consumption in 2014, a loss of between £4.03 and £5.38 million was sustained by growers. The conflict between the figures described may be more easily understood when put into the trading context. There is relatively widespread use for human consumption of beans with slightly higher levels of damage, and in these cases penalties are applied to growers for grain containing greater than 2% damage, as damaged beans are removed during processing. The degree to which this is undertaken may vary each year depending on the availability of good quality beans. Broad beans for fresh and frozen end use are rejected completely when damaged by *B. rufimanus* and tolerance for damage is zero. The presence of larvae in broad beans leads to the produce being unacceptable for human consumption. In those areas of the UK where *B. rufimanus* is present, broad beans are no longer produced. Previous areas of broad bean production included Norfolk, the Midlands and Yorkshire (British Growers Association, 2016, Personal Communication).

2.2.5 *Diapause and host-plant interaction*

Adult *B. rufimanus* enters over-wintering sites during September and October in the UK and is in reproductive diapause, although active, on emergence from *V. faba* seeds in August and September (Tran *et al.,* 1993). Kostal (2006) described the general mechanisms of diapause initiation in insects stating that in mobile stages, for instance adults, initiation is characterised by a very gradual decrease in metabolic rate, often accompanied by behavioural and physiological activities required to prepare for diapause. These might include a period of intense feeding prior to seeking a suitable microhabitat and as a consequence metabolic rate slows gradually to allow for these activities. Diapause is then maintained and metabolic rate remains low as the insects remain in arrested development, even if conditions are suitable for the continuation of development directly following the initiation stage. Although *B. rufimanus* adults are already in a state of reproductive diapause in late summer following emergence from *V. faba* grains, there is a requirement for adults to feed prior to entering overwintering sites, find suitable habitats for winter and to maintain reproductive diapause during a period when summer conditions may still exist. Although very little is known about the maintenance or true phase of diapause, developmental changes occur in *B. rufimanus* that reduce the intensity of reproductive diapause and cause the insect to become more sensitive to the stimuli that lead to termination of reproductive diapause (Tran *et al.,* 1993). Chapter 4 discusses the factors that influence the emergence of *B. rufimanus* from winter sites and the start of oviposition, hypothesising that temperature and photoperiod are the determining factors leading to emergence from overwintering sites in spring, and seeking to investigate these factors in natural conditions.

Two types of reproductive diapause termination have been proposed by Hodek (1996, 2002), one that requires specific environmental or external cues, termed tachytelic, and a second in which development at the standard rate occurs without the influence of external cues, termed horotelic. These terms have been in use for a long period and were used by Tran *et al.* (1993), who considered termination of diapause as a definite ‘end’ to reproductive diapause and resumption of activity. Hodek (1996, 2002) avoids the term ‘diapause termination’ as it has been used to indicate the whole process of diapause completion as well as to describe an unspecified final stage of diapause. Tran *et al.* (1993) found that the development of reproductive diapause termination in *B. rufimanus* was a combination of two key factors, day length and food source. Tran and Huignard (1992) and Medjdoub-Bensaad *et al.* (2007) described reproductive diapause in female *B. rufimanus* as ovariole reduction to germarium and termination of reproductive diapause was characterised by the presence of vitellogenesis in ovarioles and the presence of mature oocytes at the base of the ovarioles and lateral oviducts. In males, the termination of reproductive diapause was described by Tran and Huignard (1992) as increased diameter of the two accessory glands and the presence of secretions in the lumen.

Delobel and Delobel (2006) investigated the interactions between major European bruchid species and their host plants. The study showed that *B. rufimanus* larvae were able to feed on and complete their life-cycle in a number of wild vetch species as well as *V. faba*, possibly indicating an ability to reach sexual maturity following pollen feeding in both *Lathyrus* and *Vicia* genus. The plant species that were found to host *B. rufimanus* were *Lathyrus cicera* Linnaeus (red vetchling), *Lathyrus venetus* Miller Wholfarth (Venetian vetchling), *Vicia onobrychioides* Linnaeus (sainfoin vetch), *Vicia peregrina* Linnaeus (wandering vetch), *Vicia villosa* Roth (winter/ fodder vetch), *Vicia bithynica* Linnaeus (Bithynian vetch), *Vicia faba* Linnaeus (faba bean), *Vicia hybrida* Linnaeus (hairy yellow vetch), *Vicia lutea* Linnaeus (smooth yellow vetch), *Vicia narbonensis* Linnaeus (purple broad vetch) and *Vicia pannonica* Crantz (Hungarian vetch). The samples were collected from France, southern Italy, southern Greece, Spain and Portugal and are species known to be present in the UK (National Biodiversity Network Gateway, 2016).

Tran and Huignard (1992) and Tran *et al.*, (1993) showed that reproductive diapause termination did not end in female or male *B. rufimanus* when photoperiod was less than 16 hours. Increasing photoperiod alone did not influence the proportion of females that terminated reproductive diapause, indicating the requirement for other cues. Females required the presence of *V. faba* flowers in order to terminate reproductive diapause, although a higher proportion of males terminated reproductive diapause using the photoperiod cue and the additional presence of flowers at 16 hours photoperiod did not significantly increase male reproductive diapause termination. When photoperiod was increased to 18 hours and flowers were present there was a significant increase in reproductive diapause termination in both males and females. The consumption of *V. faba* flower pollen was found, in those studies, to be important for female reproductive diapause termination and for synchronisation of the biological cycle of the species with that of the host plant.

Tran and Huignard (1992) provided further evidence that pollen type was important in termination of female reproductive diapause. Comparative experiments showed that feeding on sterile *V. faba* flowers, or those of other plant species (*Prunus* sp. and *Coryllus avellana* Linnaeus), led to significantly reduced reproductive diapause termination in females than those fed on fertile *V. faba* flowers, although feeding on the flower pollen of *Prunus* sp. and *C. avellana* did lead to greater reproductive diapause termination than when females were fed on either sterile *V. faba* flowers or sucrose solution. Male reproductive diapause termination was slightly lower when feeding on sterile flowers or flowers of other species, but significantly higher than that of the females. The study was limited by the relatively few flowering species included, and it is not clear how many experiments were carried out or whether treatments were replicated. During the flowering period it is thought that *B. rufimanus* consumes only the pollen of *V. faba* although it is possible for vitellogenesis in adult females to be induced by a diet of other *Vicia* or *Lathyrus* species (Huignard *et al.,* 1990).

Bruce *et al.* (2011) investigated the effects of *V. faba* semiochemical compounds, and volatiles from post-diapause male *B. rufimanus,* on electro-physiological and behavioural responses of *B. rufimanus* in laboratory and field experiments. They found significant electro-physiological and behavioural responses of both males and females to the plant semiochemicals myrcene, (*R*)-limonene, (*E*)-ocimene, (*R*)-linalool, 4-allylanisole, cinnamyl-alcohol, cinnamaldehyde, (*E*)-caryophyllene and alpha-humulene. Females responded electro-physiologically to the male pheromone 1-undecene but did not respond behaviourally to the compound in field conditions. The study indicated that the attraction of the host plant *V. faba* was the primary stimulus for *B. rufimanus* activity and that the male pheromone, although stimulating responses from females, was more attractive at close range.

There have been further advances in host range prediction in recent years, including the use of mixed model equations (MME) to generate Best Linear Unbiased Predictors (BLUP), developed in response to inaccuracies in predicting host range of biological control agents. BLUP’s can be generated for species with little or no observed data, and the MME are suited to both the evaluation of test plant species and to the generation of test plant lists (Berner, 2010).

2.2.6 *Geographic distribution*

The influence of landscape and in-field features and connectivity of habitats on distribution has been well described in other insect species (Burel, 1992; Diekotter *et al.,* 2008; Zaller *et al.,* 2008). Factors that influence distribution include the level of polyphagy that insects show, their ability to disperse, the impact of semi-natural habitats on parasitic organisms, environmental conditions and crop management techniques (Rusch *et al.,* 2013). Most important are the presence of the host crop and the spatial distribution of host crops, or fragmentation of the host crop landscape (Schneider, 1999; O’ Rourke *et al.,* 2011), and environmental conditions. Stewart *et al.* (2015) listed three groups of constraints that limit the spatial distribution of species. These were tolerance to environmental conditions such as temperature and ability to adapt to those conditions, the availability of resources such as food, and interactions with other species such as predators or natural enemies. Further restrictions on spatial distribution may be caused by human activity and by the presence of physical landscape barriers such as mountain ranges or breaks in the natural habitat of the species. Stewart *et al.* (2015) also indicated that the primary influencing factor for phytophagous insect distribution is host plant distribution, although the relationship was not thought to be a simple linear relationship where increased range of host plant caused increased range of species. In many insect species the range size of the species had a weak relationship with the range size of the host plant, and was constrained, in addition to host plant presence, by other factors. Other studies have shown that distribution may be more closely associated with host plant density (Quinn *et al.*, 1997).

Cox (2007) described known global distribution of *B. rufimanus* as Finland, Sweden, Denmark, Estonia, Lithuania, Poland, France, Spain, Portugal, Belgium, Germany, Austria, Switzerland, Italy, Malta, Czechoslovakia, Hungary, former Yugoslavia, Croatia, Macedonia, Bulgaria, Greece, Turkey, Cyprus, Israel, Iran, Japan, Morocco, Libya, Algeria, Tunisia, Kenya, Angola, the USA, Trinidad and Argentina.

There have been few studies of the distribution of *B. rufimanus* in the UK, although there are existing records for the species that date back to 1850 (National Biodiversity

Network Gateway, 2016) as well as archaeological reports of *B. rufimanus* (Carrott *et al*., 1995) dating back to the Saxon or early medieval periods. The National Biodiversity Network (NBN) is a collaborative partnership that shares information from a number of organisations, exchanging biodiversity information and records. The NBN Gateway maps illustrate the distribution of *B. rufimanus* from 1850 to the present, from records obtained by specialist biodiversity recorders and from the Seed and Leaf Beetle Recording Scheme (Cox, 2007). The current distribution as represented on these maps demonstrates that *B. rufimanus* is present in most southern counties, up to Wales, the Midlands, Yorkshire and at its most northern point in Dumfries and Galloway where it was recorded in the 1890's (Figure 3.1).

Chapter 3 describes the distribution and relative abundance of *B. rufimanus* across the UK compared to the range of the host plant *V. faba* and examines the relationship between distribution of *B. rufimanus* and regional temperature.

2.3 Control and management strategies

Control of *B. rufimanus* is primarily conducted using insecticide applications at the mid-flowering and early pod-set growth stages in *V. faba* to target adults prior to oviposition. Pyrethroids have been the only active ingredients available for use in *V. faba* for the control of beetle pests in the UK since the late 1990's when organophosphate product use was phased out. Managing adult *B. rufimanus* pest attacks is difficult due to their mobility, and the lack of persistence of pyrethroids at high temperatures (Mansoor *et al.,* 2015). Pyrethroids have been widely and regularly used to control *B. rufimanus* and although there is no confirmation of resistance to these active ingredients in *B. rufimanus* in the UK, there are preliminary indications that resistance may be arising as a consequence of the lack of alternative insecticide modes of action, and repeated use of pyrethroid insecticides (L. Smart, 2015, Personal Communication). Another common beetle pest of *V. faba* in the UK, *Sitona lineatus* Linnaeus (Coleoptera: Curculionidae) (pea leaf weevil), has recently developed resistance to pyrethroid insecticides (Foster, 2015), although the specific mechanism of resistance is not yet known. Crop growth stage at application, the attraction of *V. faba* as a floral resource for beneficial insects and the difficulty of achieving complete control of *B. rufimanus* using pyrethroid insecticides, has caused a considerable dilemma for growers when planning field control measures. The impacts of pyrethroid insecticides on bees are well known and include effects on foraging behaviour (Gill and Raine, 2014), motor function, grooming and wing fanning behaviour (Oliver *et al*., 2015), as well as direct toxic contact effects, particularly in combination with ergosterol-inhibiting fungicides (azoles) (Vandame and Belzunces, 1998; Sanchez-Bayo and Goka, 2014). Continued research is required to reduce the impact of *B. rufimanus* in *V. faba* using cultural, plant breeding and alternative crop management techniques rather than chemical means.

There is evidence that legume species have specific phytotoxic effects on insects that colonise them (Holloway, 1986; Desproches *et al.,* 1995; Sandrine *et al.,* 2007). Gbaye *et al.* (2011) described the effects of temperature and phytochemical properties of some legumes on the susceptibility of three species of the genus *Callosobruchus* (Coleoptera: Chrysomelidae) to insecticides, concluding that food source effect was partially due to ancestral host, depending on the species. When the ancestral host was the same as the current host, tolerance to insecticides was higher. UK *B. rufimanus* has a very strong interaction with *V. faba* and has a host range limited to *Vicia* and *Lathyrus* genus, and it is possible that the ancestral relationship between the species has led to greater tolerance to insecticides. Gbaye *et al.* (2011) tested the three *Callosobruchus* species using malathion, an organophosphate insecticide. These are known to have improved activity as temperature increases, compared to pyrethroid insecticides which have reduced activity as temperature increases (Vandame and Belzunces, 1998). Further work by Gbaye *et al.* (2011) described the effects of geographical strain of the species of bruchid beetle *Callosobruchus maculatus* Fabricius, temperature and larval food on insecticide tolerance. The authors found that all of these factors influenced the level of tolerance to the organophosphate insecticide malathion. Although there were high levels of variability in the significance of results described between the environmental factors in the study, temperature was found to have a significant influence on the tolerance of *C. maculatus* to malathion. It was proposed that possible effects of legume phytochemical components may have contributed to the differences between the strains of *C. maculatus*. Chapter 5 seeks to further explore the relationship between temperature, crop development and pyrethroid application timing, hypothesising that temperature and crop development thresholds are required at application to provide optimum control of damage by *B. rufimanus*.

In a description of agronomic techniques that may be used to manage pest attacks in *V. faba* Stoddard *et al.* (2010) discussed site selection, crop rotation, cultivar and seed selection, sowing date and plant density as potential means to control some pests. There have been several recent studies investigating the influence of *V. faba* cultivar and sowing date on the incidence of *B. rufimanus* (Szafirowska, 2012; Seidenglanz and Hunady, 2016). These have provided some evidence that delayed sowing and later crop development may reduce the impact of *B. rufimanus* damage in *V. faba*. Thus, the effects of alternative cropping strategies, specifically sowing date, plant density and cultivar, are investigated in Chapter 6 with the aim to elucidate relationships between these factors and damage, and to investigate interactions between the factors.

Entomopathogens and volatile plant oils have been investigated for efficacy against *B. rufimanus* and *Bruchidius incarnatus* Boheman (a storage pest of *V. faba*) and have shown varying degrees of entomopathogenic, insecticidal or repellent activity (Liu *et al.*, 2006; Sabbour and E-Abd-El-Aziz, 2007; Sabbour and E-Abd-El-Aziz, 2010). The entomopathogen *Beauveria bassiana* (Balsamo) Vuillemin has shown particular promise for the control of *B. rufimanus* and a current investigation is being undertaken to study its efficacy (Bruce *et al.,* 2016). A study undertaken to investigate the usefulness of intercropping *V. faba* with phacelia showed no reductions in damage caused to the crop by *B. rufimanus* (Wnuk and Wojciechowicz-Zytko, 2010).

There exists in the UK a monitoring and forecasting system to provide decision support to growers that was developed in 2014 and hosted by Syngenta Crop Protection UK (Syngenta, 2016). The system utilises a managed network of growers that annually monitor activity of *B. rufimanus* in *V. faba*, providing the results of monitoring to the Processors and Growers Research Organisation, combined with a temperature forecasting model to aid the decision to apply insecticides (BruchidCast®).

While there is a large body of literature describing *B. rufimanus* as a pest in *V. faba*, there are still gaps in the knowledge required to manage pest incidence. These relate particularly to the conditions that cause variable levels of damage to *V. faba*, factors that lead to variable efficacy of insecticides, and alternative methods of management. The effect of temperature on activity of *B. rufimanus* is further investigated in Chapters 3, 4 and 5. The investigation of geographic distribution (Chapter 3) aimed to provide growers with a prediction of likely regional incidence and to provide improved risk assessment for regional management approaches. The aim in Chapter 4 was to investigate the factors influencing emergence of *B. rufimanus* from over winter sites and to test the hypothesis that temperature and photoperiod affect emergence. Although investigated by Tran and

Huignard (1992) and Tran *et al.* (1993), these studies were carried out in laboratory conditions and Chapter 4 investigates the effects of these factors in natural conditions.

Alternative methods of managing *B. rufimanus* were investigated in Chapter 6 and the study of sowing date, plant density and cultivar on *B. rufimanus* damage aimed to provide further advice to growers who seek alternatives to conventional insecticide use. Further observations were undertaken with the aim to improve understanding of *B. rufimanus* overwintering behaviour, and these are described in Chapter 7.

2.4 Thesis structure aims and objectives

As indicated in this chapter the thesis has a range of aims and objectives which are summarised below.

Chapter 3: **UK Distribution of *B. rufimanus***:

Aim: To examine the relationship between the distribution of *B. rufimanus* and (i) density of the host plant species, and (ii) regional temperature.

Objectives: To produce distribution maps of the *V. faba* crop and damage by *B. rufimanus;* to produce statistical analyses of the relationship between the distribution of B. rufimanus and (i) density of the host plant species, and (ii) regional temperature

Chapter 4: **Conditions required to stimulate emergence of *B. rufimanus* from overwintering sites and commencement of oviposition**:

Aim: To examine the factors that influence the emergence of B. rufimanus from winter sites and the start of oviposition.

Objectives: To identify thresholds of photoperiod and temperature when activity and oviposition by *B. rufimanus* can be detected in the field.

Chapter 5: **The influence of temperature, crop development and insecticide applications on oviposition and damage caused by *B. rufimanus* in *V. faba***

Aim: To explore the relationship between temperature, crop development and pyrethroid application timing.

Objectives: To establish whether temperature and crop development thresholds are required at application to provide optimum control of damage by *B. rufimanus*.

Chapter 6: **The influence of sowing date, cultivar and plant density on the damage caused by *B. rufimanus* in *V. faba***

Aim: To examine the effects of sowing date, plant density and cultivar, on damage.

Objectives: To analyse statistically whether (i) sowing date, (ii) cultivar, and (iii) plant density affect damage by *B. rufimanus* and crop yield.

Chapter 7: **Observations of *B. rufimanus* overwintering habitats and pollen feeding behaviour prior to overwintering**

Aim: to identify the habitats that host *B. rufimanus* during the winter period and to investigate factors that influence survival during the winter.

Objectives: to record the feeding preferences of *B. rufimanus* prior to overwintering, and to identify the habitats that host *B. rufimanus* during the winter period.

Chapter 3: UK Distribution of *B. rufimanus*

3.1 Introduction

Studies of phytophagous insects have shown that there are a number of factors that influence distribution, including physical constraints such as temperature and landscape features, host plant or resource distribution, ability of the insect species to adapt to climate or resource availability, and interactions with other species such as natural predators (Burel, 1992; Quinn *et al.*, 1997; Schneider, 1999; Diekotter *et al.,* 2008; Zaller *et al.,* 2008; O' Rourke *et al.,* 2011; Rusch *et al.,* 2013; Stewart *et al.,* 2015). Interactions between plants and their insect herbivores can be strongly influenced by regional climate and seasonal changes in temperature and precipitation (Leckey *et al.,* 2014). The geographical ranges of insect species are generally determined by their tolerances, or those of their food resources and predators, to variation in abiotic conditions (Schowalter, 2011). Global patterns of temperature and precipitation determined by interactions between latitude, atmospheric and oceanic circulation patterns, and topography, establish a regional template of physical conditions that support characteristic community types. The physiological tolerance of insects usually reflects the conditions of the biome in which they occur.

Many studies have described the likely impact of climate warming on changes in geographical distribution of insects and other organisms (Karafyllidis, 1998; Pelini *et al.,* 2010; Knell and Thackeray, 2016). It is generally accepted by the scientific community that current climate change is caused by human activity. Mean annual temperature in Central England between the 1970's and 2009 increased by 1°C (Defra, 2009), and it could be proposed that the gradual increase in populations and levels of damage caused by insect pests such as *B. rufimanus* in the UK may in part be due to the increase in temperature in the last 40 to 50 years. Projected increases in average summer temperatures in south-east England are 1.6°C (0.6-2.7°C) during the 2020s, 2.3°C (1.0-4.0°C) by the 2040s and 3.9°C (2.0-6.4°C) by the 2080s (Defra, 2009). Climate is one of the most important abiotic factors that affect species birth rate and mortality and hence species richness (Baldacchino *et al.,* 2017), and insects are particularly responsive to climate change because much of their life cycle is influenced by temperature. However, they are also affected by the quality and availability of plants as food resources (Pelini *et al.,* 2010). The 'ideal free distribution' theory is used to describe how organisms are distributed across several patches of resources, and states that the number of organisms present in a patch is proportional to the quantity and quality of resource available (Williams *et al.,* 2013). Species distribution may change as habitats at poleward latitudes or higher altitudes become more suitable for colonisation, and as species shift they may become established in these areas (Pelini *et al.,* 2010). Dispersal to new geographic areas is an expected consequence of climate change (Battisti and Larsson, 2015). Spatial scale, timescale over which change takes place and difficulty in observing individual insects that have extended their range, often leads to difficulties obtaining data on the dynamics of the distribution range of insects. For management of insect pests in agriculture, it is important to understand whether range expansion of an insect will lead to it becoming an agricultural pest in the new area, and if so, whether the dynamics will be similar to that in the historic range (Battisti and Larsson, 2015). For instance, it is important to know whether *B. rufimanus* could survive in Scotland, the only area now commercially producing broad beans for the frozen market, and if so, the likely magnitude and frequency of damage to those crops.

Differences in the response of species to climatic changes can be important when the plant-host relationship is specific, as plants are not always able to adapt to climatic change as quickly as the insects that colonise them and spatial mismatch of coloniser and host occurs (Schweiger *et al.,* 2008). The response of insect communities to climate may be subject to plant-mediated effects, which may or may not outweigh the direct effect of temperatures on insects (de Sassi *et al.,* 2012).

Many plants display phenological responses to climate warming (Cleland *et al.,* 2012) and it is possible that field beans will respond to climate changes by displaying variation in the timing of crop growth stage events such as flowering and pod set. As climate warms, these phenological events may occur either earlier or later in the growing season, as changes in soil and air temperature influence the onset of plant growth. Autumn sown *V. faba* requires a vernalisation period, a sufficient period at low temperature to prevent the onset of flowering during the winter. The vernalisation requirement of winter beans is about 30 days, and although winter beans respond to vernalisation at 4°C, unvernalised plants eventually flower at a higher node (Link *et al*., 2010). The varying effects of climate change cause difficulty predicting changes in environmental conditions for bean cultivation, and drought and higher temperature may lead to conditions becoming less suitable for cultivation in some regions of the UK. A simulation model for faba bean, CROPGRO-Fababean

(Confalone *et al.,* 2010), shows that the duration of phenological phases before flowering is controlled by temperature and photoperiod, and after flowering faba bean phenology is responsive only to temperature. The effects of climate on *V. faba* phenological development may have wider impact regarding the impact of pests on crop production. Abiotic stress conditions such as drought, high and low temperature may influence the occurrence and spread of insects and may result in minor pests becoming potential threats in future (Legreve and Duveiller, 2010). Chapter 6 describes an experiment conducted in 2015 to evaluate the effect of different sowing dates of *V. faba* on damage caused by *B. rufimanus*, and results indicated that earlier spring sowing offered better host-plant synchronisation for *B. rufimanus* colonisation, leading to greater levels of damage to harvested seed.

Temperature is thought to be one of the key factors influencing the survival and distribution of *B. rufimanus*, although there have been no previous studies investigating the relationship between regional temperature and *B. rufimanus* distribution in the UK. Biological records of adult *B. rufimanus,* available from the National Biodiversity Network (NBN) Gateway (2016), provide a good indication of the historic and present distribution of the species. NBN Gateway maps represent the results of records made by specialists including those working on behalf of wildlife charities and non-governmental organisations, local environmental record centres, research institutes, students, government agencies and members of the public and are accepted as reliable. Data from the Seed and Leaf Beetle Recording Scheme (Cox, 2007) was incorporated into NBN Gateway records in 2008 and, combined with other records, provides a picture of distribution of *B. rufimanus* from pre-1900 to the present (Figure 3.1). The first record of *B. rufimanus* on the NBN database was in 1863 and the only recorded incidence in Scotland dates to the 1890’s. Archaeological studies identified *B. rufimanus* from Saxon and Early Medieval sites in the UK (Carrott *et al.,* 1995).

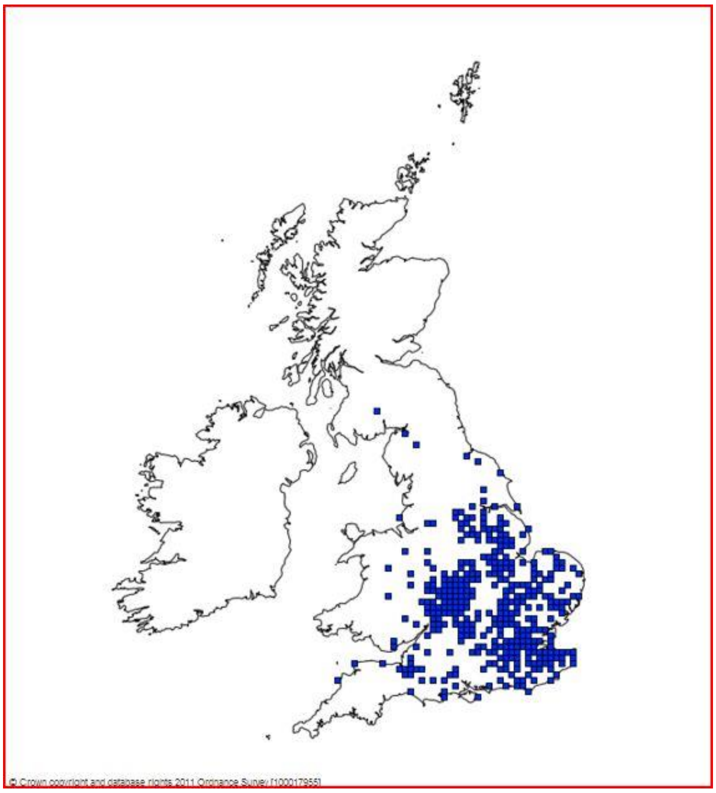


Figure 3.1: UK distribution of *B. rufimanus* from records available from the National Biodiversity Network (NBN) Gateway, 1863-2016.

The current prediction service for growers seeking to manage *B. rufimanus* in the UK uses a temperature threshold of two consecutive days when maximum daily temperature reaches at least 20°C at the time of first pod formation (Syngenta, 2016). First pod formation is described as lower pods being 2cm long and no longer encased in flowers. When these events occur together, first insecticides are applied to crops. This recommendation is also used in France (Charbonnaud *et al.*, 2016). While it is generally accepted that this provides growers with an indication of when insecticide applications will be most effective, in some years control of *B. rufimanus* fails to a large degree, or variable control is achieved, despite many growers in southern areas of the UK using this recommendation. Discussion amongst growers and agronomists suggests several reasons why this may be the case. Temperature from emergence to the date of first pod may influence the fecundity of *B. rufimanus*, as may temperature after the formation of first pods, during the period when oviposition is greatest. Poor targeting of insecticides may occur due to dense canopy preventing penetration of substances to the lower part of the plant where a large part of the damage occurs (Chapter 2, Subsection 2.2.2). In some instances, growers reduce the water volume used to dilute insecticides at application to the minimum required according to the label recommendation. This allows applications to be carried out more quickly but may lead to a reduction of efficacy of insecticides, particularly for contact acting insecticides applied to concealed pests (HSE, 2017). The combination of these factors, and the high mobility of *B. rufimanus,* may lead to suboptimal efficacy of insecticide applications.

This study seeks to elucidate the relationship between temperature and damage to harvested seed using data representing the entire UK *V. faba* production area. The study aims also to describe the current status of the distribution of *B. rufimanus* in the UK, and to provide an indication of regions that are at greatest risk. A useful tool for *V. faba* growers would be the ability to predict conditions leading to greater levels of crop damage, providing information to allow them to better manage crops on a regional or local scale. The relationships between distribution of *B. rufimanus* and average mean regional temperature for April and May, the period of emergence of *B. rufimanus* from overwintering sites, and June and July, the key period for pod formation and pod filling in *V. faba,* and oviposition by *B. rufimanus,* are investigated. Data for grain damage was gathered with the assistance of Frontier Agriculture Ltd. (Frontier Agriculture Ltd., 2015, Personal Communication), one of the largest grain traders in the UK, and represents the entire geographical area of *V. faba* production in the UK (R. Vickers, 2017, Personal Communication). Each sample represents an individual crop or farm for all *V. faba* traded by Frontier during the years 2008, 2010, 2011, 2012 and 2015, totalling 6398 crops or farms during that period, representing approximately 25% of total UK production (R. Vickers, 2016, Personal Communication). The overall aim is to provide a better understanding of the distribution of this major insect pest of *V. faba* and to assist growers with decision making based on the likely incidence of *B. rufimanus* in their region.

3.2 Methods

Data analyses and mapping were carried out using raw data supplied by Frontier Agriculture Ltd. for the years 2008, 2010, 2011, 2012 and 2015. The datasets contained information for all *V. faba* grain samples received at their distribution centres throughout the UK, taken as the bulk bean loads arrived at depots. Samples of 100 grains were taken from each lorry load of dry harvested beans as they arrived at distribution centres and assessed for damage caused by *B. rufimanus*. Each lorry load represented either an individual crop or an individual farm producing *V. faba*. Damage was recorded as number of damaged grains, and each sample recorded and archived with a postcode reference per sample consisting of the first half of the postcode (e.g. PE8). Datasets were not provided for 2009, 2013 and 2014, although summary regional data were available for those years. In total, data for 6398 samples were analysed.

Data were handled in Microsoft Office Excel®. Postcode data by sample were converted to grid reference data using an online batch converter tool (Grid Reference Finder, 2017). Met Office datasets for mean daily temperature were downloaded from the data server of the Centre for Environmental Data Analysis (Met Office, 2017). The datasets contain daily observations of temperature interpolated to a uniform 5 kilometre grid resolution covering the period 1960 to 2016. The input station data originate from the Met Office Integrated Data Archive System and provide observations relating to periods 0900 hours to 0900 hours (Met Office, 2017). Temperature and recorded *B. rufimanus* damage data were combined at grid reference level. Mean *B. rufimanus* damage was calculated at postcode district level using grid reference, including count of samples per postcode district.

Data were imported, with assistance, into Stata® version 14 (T. Robertson, 2017, Personal Communication) in Comma Separated Value files at the level of postcode district to create maps of the distribution of the *V. faba* samples evaluated. Maps were created by creating scatterplots, using the co-ordinates of all UK Meteorological Stations, to visually compare mean damage per postcode district and county for the cultivars Wizard, an autumn sown cultivar, and Fuego, a spring sown cultivar, with average mean daily temperature for June and July for each year. The proportion of damage and mean temperature were differentiated by colour to provide an illustration of the extent and intensity of *B. rufimanus* damage per year across the UK, compared to the average mean daily temperature for June and July. Unknown cultivars within the dataset were not mapped, as it could not be determined whether they were autumn or spring-sown cultivars. The cultivars Fuego and Wizard were illustrated separately to indicate the difference in damage levels between autumn sown and spring sown crops.

Multiple regression analyses were carried out to determine relationships between mean *B. rufimanus* damage and average mean daily temperature for months April, May, June and July for each year, at sample level for cultivars Fuego and Wizard. These months were selected for analysis, being the optimum period of *B. rufimanus* activity from adult emergence from overwintering sites during April and May (Chapter 4) until all pods have formed and filled on *V. faba* plants. Multiple regression analysis was carried out on all data at sample level, including unknown cultivars, to determine relationships between mean *B. rufimanus* damage and average mean daily temperature for months April, May, June and July for all years to evaluate between year influences of temperature.

Multiple regression analysis was carried out to examine the relationship between the number of days when specific temperature was experienced and *B. rufimanus* damage for each month April to July at sample level for all years. Categories tested were number of days when maximum daily temperature was from 17.5°C to 20°C, 20°C to 22.5°C, 22.5°C to 25°C and above 25°C to evaluate whether the number of days in a particular temperature range provided a better indication of likely damage compared to average mean daily temperature per month. The current recommendation uses a temperature threshold to guide insecticide applications, of two consecutive days when maximum daily temperature reaches 20°C prior to application.

Regression analysis was carried out to determine whether there was a relationship between the number of samples collected and *B. rufimanus* damage, at the level of postcode district and county, to evaluate whether the dataset could provide an indication of the effect of cropping density on damage.

3.3 Results

Distribution of *V. faba* in individual years showed small changes between 2008 and 2015. Samples were collected from the far West of Wales until 2011 but not in 2012 or 2015 (Figures 3.4 to 3.8). In 2008 and 2012, fewer samples were collected. 735 samples were collected in 2008, 1282 in 2010, 1284 in 2011, 881 in 2012 and 2216 in 2015. Average temperature and rainfall data for the UK is shown in Appendix B, Table XI (Met office a, 2016; Met Office b, 2016). In 2008 and 2012 rainfall was above the 30 year long-term average (1971 to 2000), particularly during the crop growth stages when pods were forming and filling (June and July). Mean annual rainfall in 2015 was also higher than the 30 year average but not during the stages of pod formation and filling (Appendix B, Table XI; Met Office a, 2016; Met Office b, 2016).

Maps of the distribution of *B. rufimanus* damaged grain, using the data collected in 2008, 2010, 2011, 2012 and 2015, were produced, with assistance, in Stata® version 14 for cultivars Wizard and Fuego (Figures 3.4 to 3.8) (T. Robertson, 2017, Personal Communication). Samples were mapped for comparison with average mean daily temperature in June and July by postcode district and show mean *B. rufimanus* damage and average mean daily temperature for each postcode district in which they occur (Figures 3.4 to 3.8).

Table 3.1: Mean percent *B. rufimanus* damage, recorded as number of damaged grains per 100 grain sample, across the UK for all cultivars and samples, with standard error of mean and number of samples per year. Date of first pod formation estimated from observations of spring *V. faba* crops at a site in Norfolk (Grid reference TF552148).

Year	Mean *B. rufimanus* damage UK	Number of samples collected	Date of first pod formation
2008	1.75 ± 0.15	735	16 June
2010	3.92 ± 0.13	1282	23 June
2011	4.98 ± 0.15	1284	2 June
2012	4.10 ± 0.18	881	10 June
2015	2.97 ± 0.09	2216	15 June

Damage intensity varied between years and there were low levels of damage in 2008 (Table 3.1, Figures 3.2 and 3.3) across the UK with only three postcode districts in the East and South-East with greater than 10% damage (Appendix B, Table I). Mean percent damage for the whole of the UK in 2008 was 1.75% (Table 3.1). Intensity of damage for both cultivars Wizard and Fuego appeared to increase in 2010, 2011 and 2012 (Table 3.1, Figures 3.2 to 3.8), particularly in the East, South-East and Midlands, and declined in 2015 (Table 3.1, Figures 3.2, 3.3 and 3.8). The ten counties from which the greatest number of samples was collected were Lincolnshire, Suffolk, Oxfordshire, Norfolk, Essex, Cambridgeshire, North Yorkshire, Hampshire, Wiltshire and Northamptonshire (Appendix B, Tables VI to X).

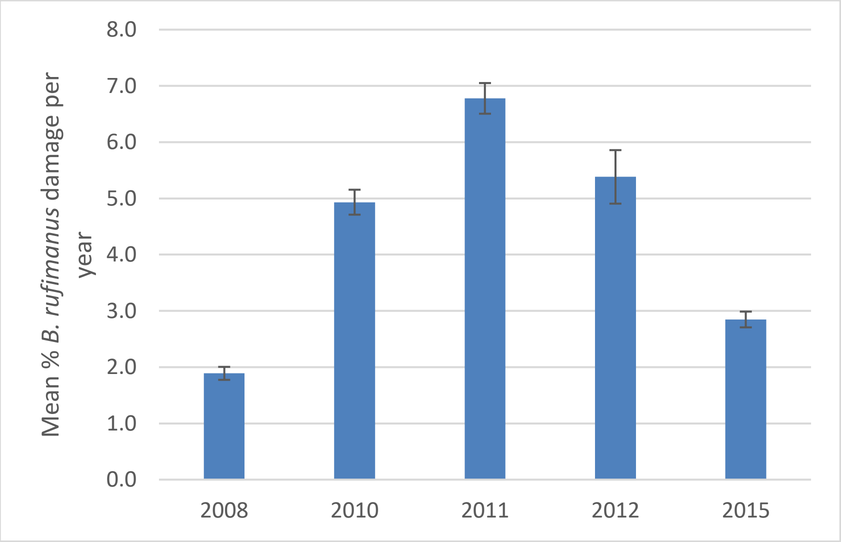


Figure 3.2: Mean damage, recorded as number of damaged grains per 100 grain sample, per year for cultivar Wizard, with error bars showing standard error.

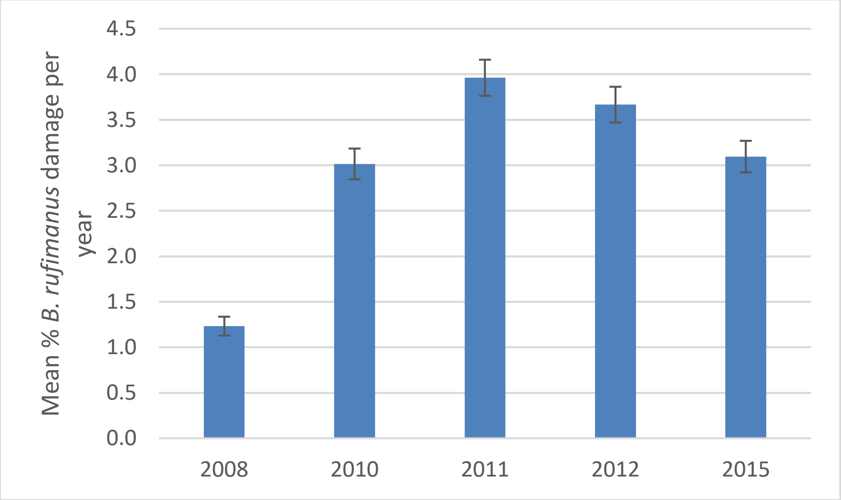


Figure 3.3: Mean damage, recorded as number of damaged grains per 100 grain sample, per year for cultivar Fuego, with error bars showing standard error.

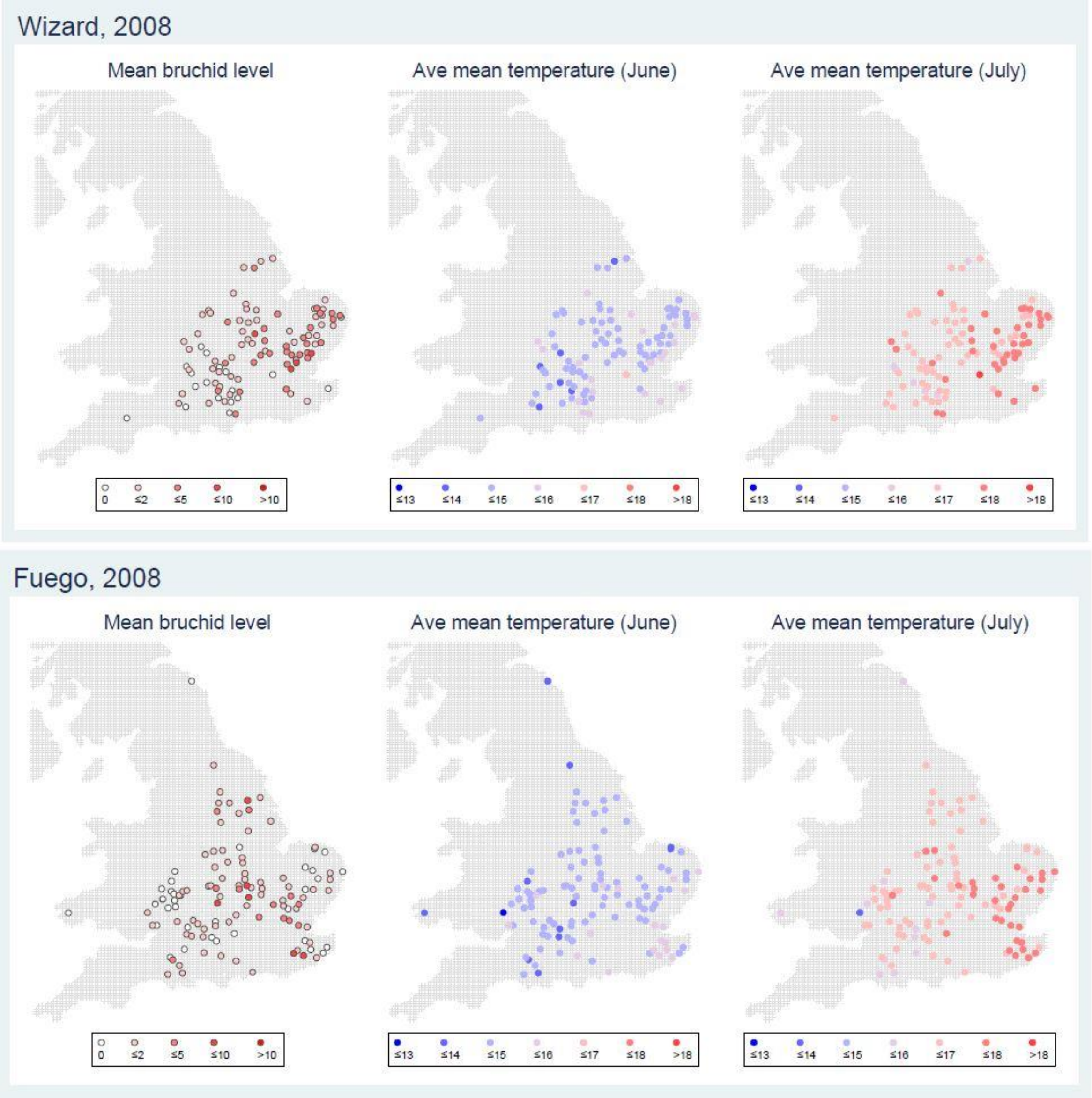


Figure 3.4: Distribution of *V. faba* grain samples, mapped by cultivar (Wizard autumn sown *V. faba* and Fuego spring sown *V. faba*), showing damage caused by *B. rufimanus* together with average mean daily temperature in June and July in 2008.

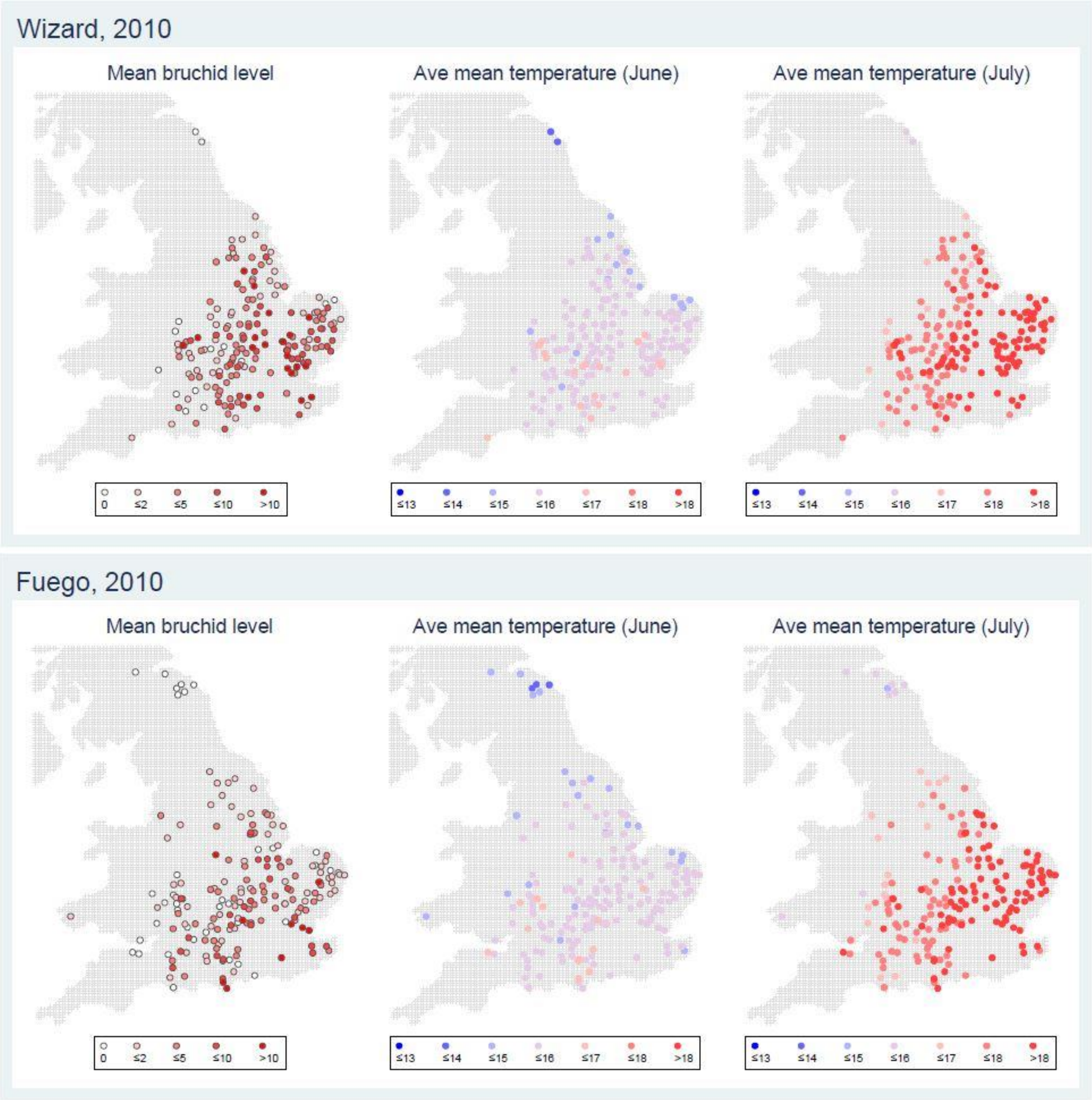


Figure 3.5: Distribution of *V. faba* grain samples, mapped by cultivar (Fuego spring sown *V. faba* and Wizard autumn sown *V. faba*), showing damage caused by *B. rufimanus* together with average mean daily temperature in June and July in 2010.

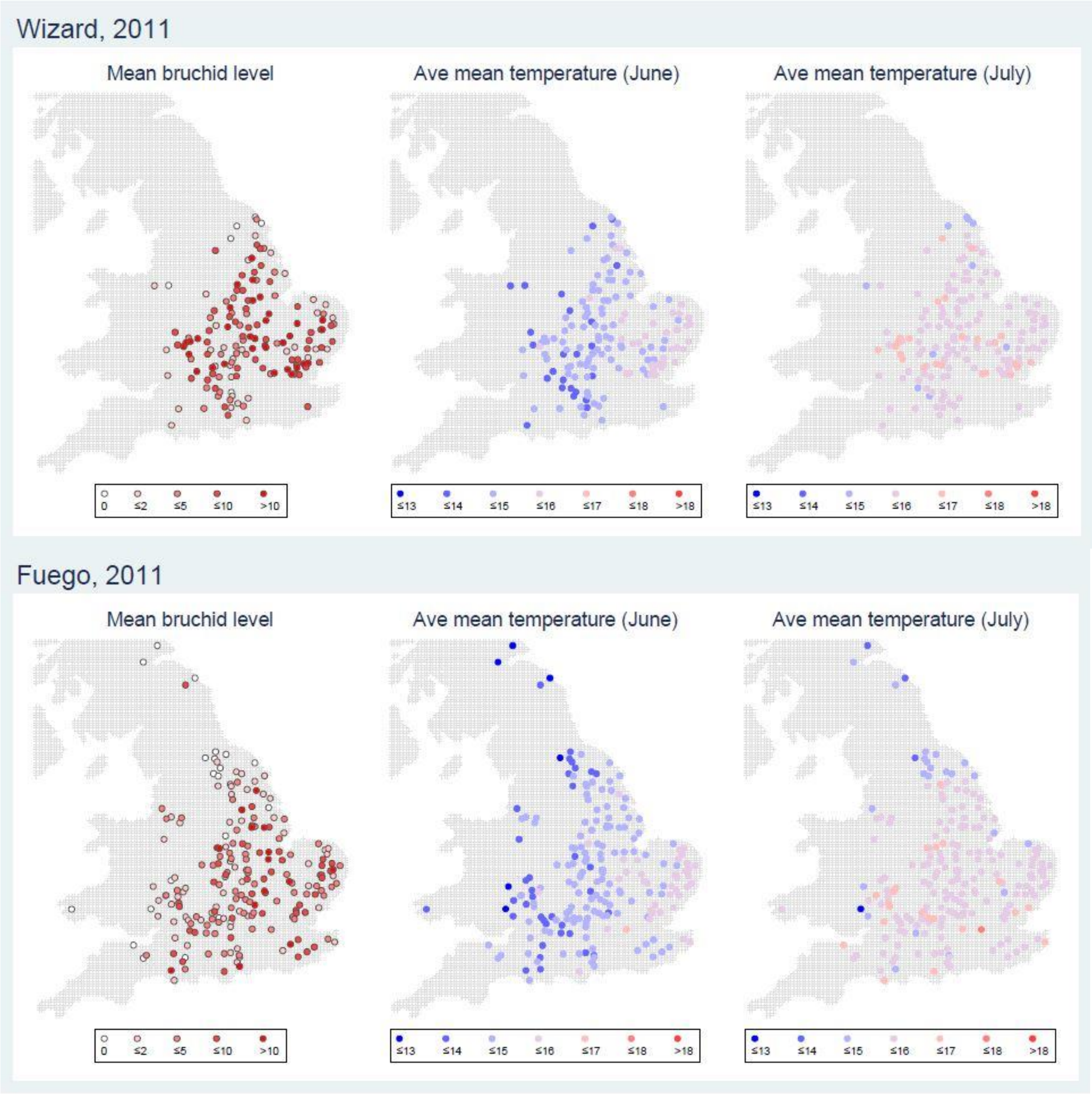


Figure 3.6: Distribution of *V. faba* grain samples, mapped by cultivar (Wizard autumn sown *V. faba* and Fuego spring sown *V. faba*), showing damage caused by *B. rufimanus* together with average mean daily temperature in June and July in 2011.

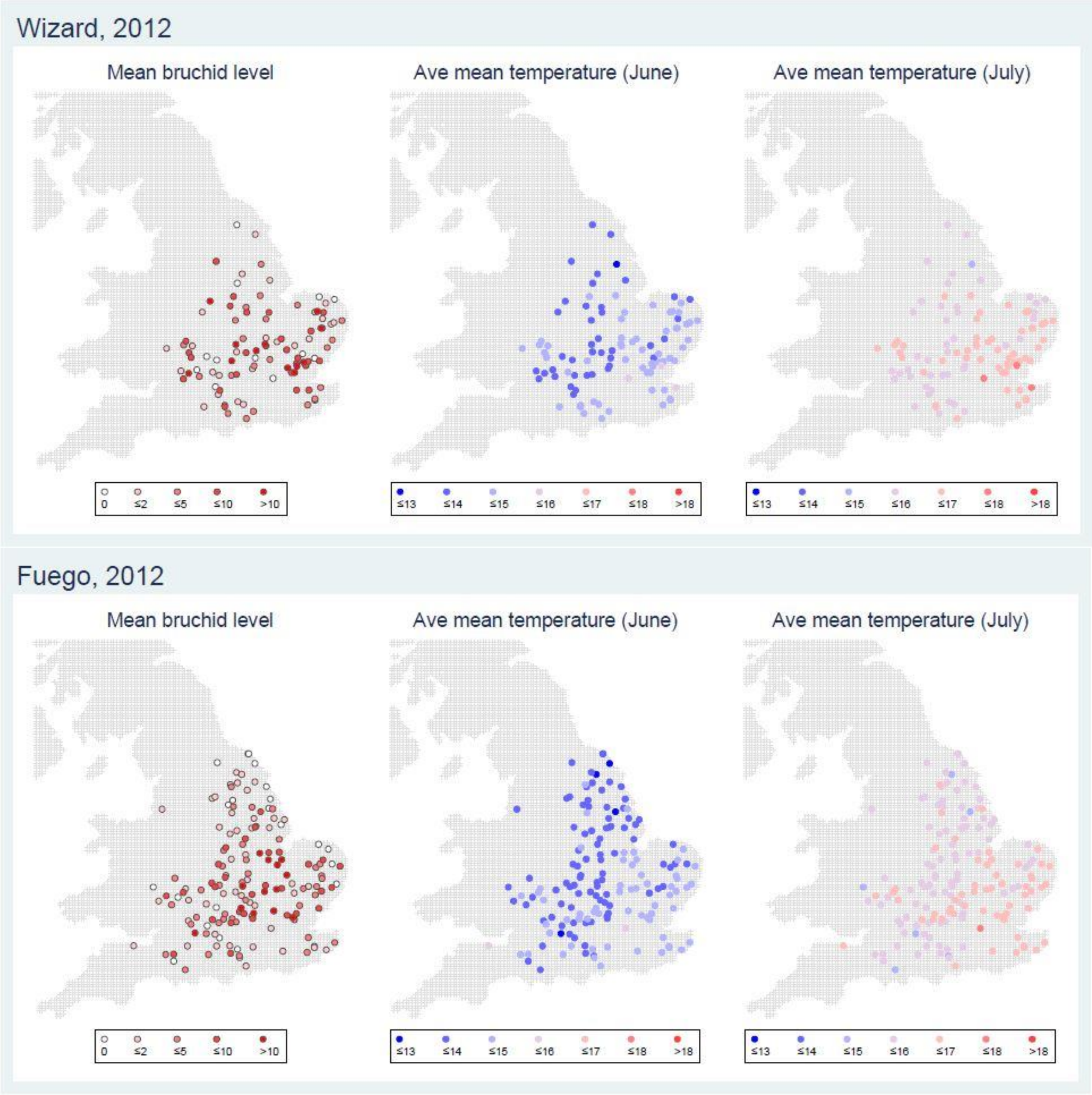


Figure 3.7: Distribution of *V. faba* grain samples, mapped by cultivar (Wizard autumn sown *V. faba* and Fuego spring sown *V. faba*), showing damage caused by *B. rufimanus* together with average mean daily temperature in June and July in 2012.

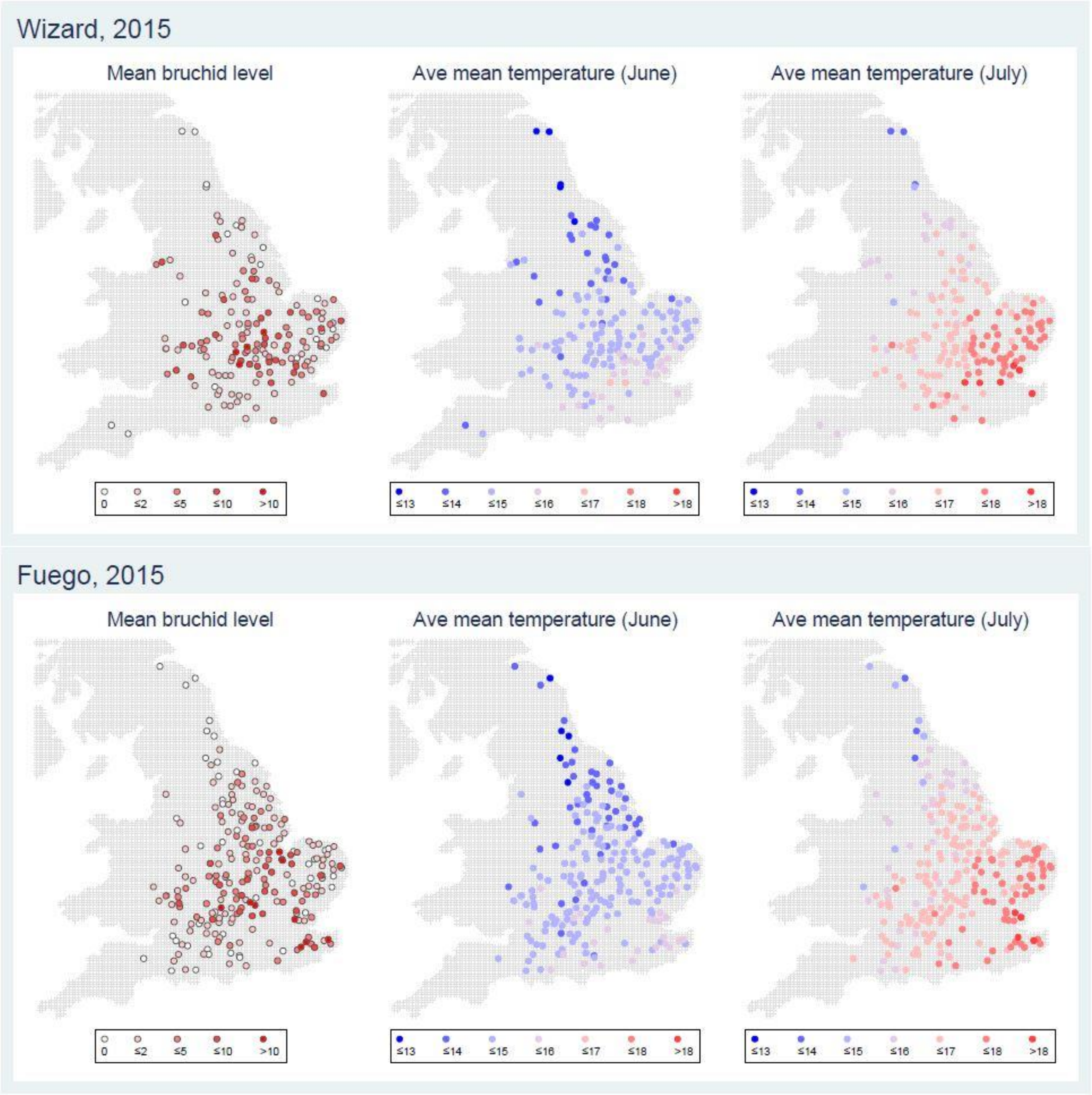


Figure 3.8: Distribution of *V. faba* grain samples, mapped by cultivar (Wizard autumn sown *V. faba* and Fuego spring sown *V. faba*), showing damage caused by *B. rufimanus* together with average mean daily temperature in June and July in 2015.

It was not possible to infer or assume differences in regional *V. faba* cropping intensity from the number of samples analysed for each year. Regression analysis for all years showed that the number of samples collected per postcode district was not related to mean *B. rufimanus* damage per postcode district (R^2=0.00, F(1,2142) = 11.38, p=0.38), and number of samples per county was only weakly related to mean *B. rufimanus* damage per county (R^2=0.13, F(1,61) = 9.19, p<.01), indicating that the dataset used here is unsuitable for inferring a relationship between cropping density and level of *B. rufimanus* damage. Number of samples at county level explained only 13% of the variability in damage caused by *B. rufimanus.*

Multiple regression analyses of data by year for *B. rufimanus* damage caused to cultivars Wizard and Fuego, at sample level, compared to average mean daily temperature in April, May, June and July showed that average mean daily temperature explained between 3 and 10% of the variability in *B. rufimanus* damage per year (Table 3.2). Significant interactions for cultivar and year are shown in Table 3.3. Significant positive regression coefficients were found in most cases, apart from 2015, for June and July average mean daily temperature when compared to *B. rufimanus* damage at sample level for both cultivars Wizard and Fuego (Table 3.3). Significant negative regression coefficients were found for April and May average mean daily temperature when compared to *B. rufimanus* damage at sample level for both cultivars Wizard and Fuego (Table 3.3).

Table 3.2: Multiple regression statistics for the relationship between average mean daily temperature in April, May, June and July and *B. rufimanus* damage caused to cultivars Wizard and Fuego in years 2008, 2010, 2011, 2012 and 2015.

Regression statistics	Cultivar Fuego	Cultivar Wizard
2008	R^2=0.10, F(4,322) = 9.19, p<.0001 N=327	R^2=0.12, F(4,272) = 9.45, p<.0001 N=277
2010	R^2=0.09, F(4,531) = 12.55, p<.0001 N=536	R^2=0.13, F(4,558) = 20.28, p<.0001 N=563
2011	R^2=0.07, F(4,599) = 11.22, p<.0001 N=604	R^2=0.11, F(4,458) = 14.17, p<0.0001 N=463
2012	R^2=0.10, F(4,459) = 12.50, p<.0001 N=464	R^2=0.03, F(4,248) = 1.76, p = 0.14 N=253
2015	R^2=0.07, F(4,815) = 16.19, p<.0001 N=820	R^2=0.09, F(4,480) = 11.38, p<.0001 N=485

Table 3.3: Statistically significant interactions between average mean daily temperature in April, May, June and July and *B. rufimanus* damage caused to cultivars Wizard and Fuego in years 2008, 2010, 2011, 2012 and 2015.

Factor	Regression Coefficient	Standard Error	t stat	P-value	Lower 95%	Upper 95%
Intercept Fuego 2008	-13.11	4.09	-3.20	<.01	-21.16	-5.06
Av. mean daily temperature Fuego April 2008	-2.57	0.55	-4.68	<.0001	-3.65	-1.49
Av. mean daily temperature Fuego June 2008	3.36	0.85	3.96	<.0001	1.69	5.03
Intercept Wizard 2008	-19.50	7.19	-2.71	<.01	-33.66	-5.35
Av. mean daily temperature Wizard July 2008	1.55	0.58	2.68	<.01	0.41	2.70
Intercept Fuego 2010	-33.85	5.74	-5.89	<.0001	-45.13	-22.57
Av. mean daily temperature Fuego June 2010	3.48	0.90	3.86	<.001	1.71	5.25
Intercept Wizard 2010	-44.17	8.04	-5.50	<.0001	-59.96	-28.38
Av. mean daily temperature Wizard May 2010	-5.82	1.45	-4.01	<.0001	-8.68	-2.97
Av. mean daily temperature Wizard June 2010	5.48	1.13	4.84	<.0001	3.26	7.71
Intercept Fuego 2011	-32.79	6.38	-5.14	<.0001	-45.32	-20.25
Av. mean daily temperature Fuego June 2011	3.00	1.01	2.99	<.01	1.03	4.98
Intercept Wizard 2011	-74.55	11.35	-6.57	<.0001	-96.84	-52.25
Av. mean daily temperature Wizard May 2011	-6.22	2.05	-3.03	<.01	-10.25	-2.19
Av. mean daily temperature Wizard June 2011	5.97	1.71	3.50	<.001	2.62	9.32
Av. mean daily temperature Wizard July 2011	3.71	1.36	2.73	<.01	1.03	6.38
Intercept Fuego 2012	-47.99	9.95	-4.82	<.0001	-67.54	-28.44
Av. mean daily temperature Fuego July 2012	3.02	1.24	2.43	<.05	0.58	5.46
Intercept Fuego 2015	-24.63	4.56	-5.40	<.0001	-33.59	-15.68
Av. mean daily temperature Fuego May 2015	2.25	1.06	2.13	<.05	0.17	4.32
Av. mean daily temperature Fuego June 2015	-3.03	1.30	-2.33	<.05	-5.58	-0.48
Av. mean daily temperature Fuego July 2015	1.91	0.79	2.42	<.05	0.36	3.47
Intercept Wizard 2015	-16.46	3.58	-4.60	<.0001	-23.48	-9.43
Av. mean daily temperature Wizard July 2015	1.87	0.53	3.55	<.001	0.84	2.91

Multiple regression analyses showed that for every increase of 1°C in average mean daily temperature in June, increase in *B. rufimanus* damage ranged from 3 to 5.48 damaged grains per sample by year (100 grains were examined for damage per sample), except in 2015 (Table 3.3). Multiple regression analyses showed that for every increase of 1°C in average mean daily temperature in July, increase in *B. rufimanus* damage ranged from 1.55 to 3.71 damaged grains per sample by year (100 grains were examined for damage per sample) (Table 3.3). Temperature alone was considered in this model.

Multiple regression analysis for all years and all cultivars, including unknown cultivars, carried out at sample level, showed that average mean temperature per month explained 5% of the variation in *B. rufimanus* damage ($R^2=0.05$, F(4,6393) = 86.48, p<.0001), with increasing temperature from April to July causing a small increase in damage (Table 3.4). The greatest influence of temperature occurred in June for all years and for every 1°C increase in average mean daily temperature in June, *B. rufimanus* damage increased by 0.53 damaged grains per sample (Table 3.4). For every 1°C increase in average mean daily temperature in April, *B. rufimanus* damage increase by 0.48 damaged grains per sample (Table 3.4).

Table 3.4: Regression statistics and statistically significant interactions between average mean daily temperature in April, May, June and July and *B. rufimanus* damage caused to all samples and cultivars for all years.

Regression statistics
$R^2 = 0.05$, F(4,6393) = 86.48, P<.0001 Number of observations = 6398

	Regression Coefficient	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	-17.31	1.35	-12.81	<.0001	-19.96	-14.66
Av. mean daily temperature April	0.48	0.05	9.08	<.0001	0.38	0.58
Av. mean daily temperature May	0.28	0.07	4.07	<.0001	0.15	0.42
Av. mean daily temperature June	0.53	0.20	2.70	<.001	0.14	0.91
Av. mean daily temperature Jul	0.32	0.13	2.49	<.05	0.07	0.57

Multiple regression analysis was used to determine whether the number of days when temperature was within a particular category (15-17.5°C, 17.5-20°C, 20-22.5°C, 22.5-25°C and greater than 25°C), analysed by month, influenced the levels of *B. rufimanus* damage (Table 3.5).

Table 3.5: Regression statistics and statistically significant interactions between number of days when average mean daily temperature in April, May, June and July was between 17.5 and 20°C, 20 and 22.5°C, 22.5 and 25°C and greater than 25°, and *B. rufimanus* damage caused to all samples and cultivars for all years.

Regression statistics

$R^2 = 0.08$, F(16,6381) = 35.49, P<.0001 Number of observations = 6398

	Regression Coefficient	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	0.57	0.76	0.75	0.45	-0.92	2.06
No. days max. daily temp. 17.5-20°C May	-0.12	0.03	-4.10	<.0001	-0.18	-0.06
No. days max. daily temp. 17.5-20°C June	0.08	0.03	2.51	0.01	0.02	0.14
No. days max. daily temp. 20-22.5°C April	0.18	0.06	3.16	<.01	0.07	0.28
No. days max. daily temp. 20-22.5°C May	-0.08	0.04	-2.13	<.05	-0.15	-0.01
No. days max. daily temp. 20-22.5°C June	0.11	0.04	3.15	<.01	0.04	0.18
No. days max. daily temp. 20-22.5°C July	-0.08	0.04	-2.06	<.05	-0.16	-0.00
No. days max. daily temp. 22.5-25°C April	0.41	0.09	4.69	<.0001	0.24	0.58
No. days max. daily temp. 22.5-25°C May	0.19	0.05	3.97	<.0001	0.10	0.28
No. days max. daily temp. 22.5-25°C June	0.22	0.04	5.36	<.0001	0.14	0.30
No. days max. daily temp. >25 °C April	2.56	0.25	10.43	<.0001	2.08	3.05
No. days max. daily temp. >25°C May	0.14	0.07	2.08	<.05	0.01	0.27
No. days max. daily temp. >25°C June	0.19	0.04	4.86	<.0001	0.11	0.26

The analysis showed that for every additional day when temperature was between 22.5°C and 25°C, and greater than 25°C, there was an increase in *B. rufimanus* damage

(Table 3.5) and the greatest influence on damage was seen when temperature in April exceeded 25°C, so that for each additional day above 25°C in April, damage increased by 2.56 grains per sample.

3.4 Discussion

Figures 3.2 to 3.6 show the incidence of *B. rufimanus* damage to *V. faba* grain across the UK compared to average mean daily temperature in June and July. The maps provide an illustrative guide for growers to assess the regional risk of incidence and severity of one of the major crop pests of *V. faba* in the UK. Although this is a comprehensive data set comprising 6,398 samples in total for the years 2008, 2010, 2011, 2012 and 2015, certain limitations of the data and the analyses must be considered when evaluating the value of the maps and analyses. Frontier Agriculture Ltd. is one of the largest traders of *V. faba* in the UK, trading approximately 25% of UK grain (R. Vickers, 2016, Personal Communication). Therefore, 75% of UK *V. faba* grain is traded by other organisations, and while the maps present a useful visual guide to for the distribution of *B. rufimanus*, it is possible that additional analysis of all UK crops would further elucidate the relationship between temperature and *B. rufimanus* incidence, and damage to crops. The data analysed here does represent the entire geographical extent of *V. faba* production in the UK (R. Vickers, 2017, Personal Communication), and a larger dataset may not improve overall knowledge of distribution if unknown factors are present that affect damage but cannot be quantified, such as the number of insecticide applications made in different regions. Maps of the distribution of *B. rufimanus* however, have not been previously available to growers.

It is likely that the use of commercial *V. faba* crop data alone provides a somewhat limited representation of the distribution of *B. rufimanus,* and that populations at the margins of its range may survive in gardens and in the wild. NBN Gateway maps, and information about the location of NBN records, indicated that *B. rufimanus* was recorded in wildlife areas and not crop areas, supporting this possibility. *V. faba* has a wider geographical range than *B. rufimanus,* and Figures 3.4 to 3.8 show the presence of *V. faba* samples that contained no damage from *B. rufimanus.*

In 2008 and 2012, fewer samples of *V. faba* were analysed, and failure to establish crops due to adverse weather may explain some of the reduction in samples collected, as well as intentional area reduction on the part of growers. Following Common Agricultural

Policy reform in 2014 crop area expanded by approximately 50% in 2015 (EUROSTAT, 2016). The reform allowed nitrogen-fixing crops, including peas and beans, to be eligible as Environmental Focus Areas within the Basic Payment Scheme (European Commission, 2016), a rural grants and payment scheme supporting European agriculture.

Regression analyses showed no relationship between the number of samples collected by postcode district, and a weak relationship between the number of samples collected per county, and mean percent *B. rufimanus* damage. This indicated that the data does not give a good representation of the density of cropping across the UK. The maps indicated that for many areas of the UK the presence of *V. faba* crops is accompanied by the presence of *B. rufimanus,* and that there are fewer areas where *V. faba* is produced and *B. rufimanus* is not present. Scotland remains free of *B. rufimanus*. Cropping intensity may not be the major constraining or enabling factor for decreased or increased activity of *B. rufimanus* in the UK. Stewart *et al.* (2015) postulated that range size is determined in phytophagous insects by multiple factors and that although the range of a species cannot exceed that of its host, other factors may further constrain distribution to smaller areas than that of the host, in particular climate and land use change. The range of *B. rufimanus* has changed little in the UK since the 1890's, although population intensity may have increased as it became a major crop pest in the early 1990's, and records of presence on wild hosts were much greater by the end of the 20th Century and early 21st Century (National Biodiversity Network Gateway, 2016). When first recorded in 1863, no records for *B. rufimanus* were made north of the Bristol Channel, although archaeological evidence documents its presence further north in Lincoln long before this period (Carrott *et al.* 1995), and in 1890 it was recorded in the Midlands. By 1900 the only recorded incidence in Scotland had been made. This relatively short period of expansion is characteristic of a species with good dispersal ability, and indicates that climate and trophic resources were not limiting. Methodological constraints should be considered when examining data from recording schemes, however, as they may give incomplete geographic coverage, are prone to under-recording (Stewart *et al.,* 2015), or simply may not exist before a certain period. Added to this is the likelihood that record submission has become better and more extensive over recent years.

Multiple regression analyses carried out to examine the relationships between mean percent *B. rufimanus* damage and average mean daily temperature in April, May, June and

July showed variation in regression correlations for all years. The influence of average mean daily temperature per month differed between the cultivars Wizard and Fuego (Table 3.2 and 3.3) and the level of damage to the cultivar Wizard, an autumn sown cultivar, was higher than that caused to Fuego, a spring sown cultivar (Figures 3.2 and 3.3).

Multiple regression analyses should be considered estimates, and associated test statistics and p-values as indicative of a possible effect, to be taken with caution. Overall, regression coefficients were significant and higher for June in most years. This may be explained in part by crop growth stage of *V. faba* during June. Table 3.1 shows estimates of the date on which first pods were formed in *V. faba* based on crop observations at a site in Norfolk where spring sown *V. faba* was grown. For 2008, 2010, 2012 and 2015 first pod formation occurred from mid-June onwards and mid-June is considered the usual time of first pod formation in spring sown *V. faba* in the UK. Winter sown *V. faba* may form pods up to two weeks earlier, but this is not always the case and pod formation in winter and spring sown field beans may occur at the same time. *B. rufimanus* females are synchronised with their host plant, *V. faba*, and oviposit only onto pods as they form. Oviposition starts when first pods are formed and may be dependent on temperature (Chapters 4 and 5). In 2011 the date on which first pods were formed was significantly earlier than in most years and occurred on 02 June. This may explain why mean daily temperature during June had a greater influence on mean percent damage caused by *B. rufimanus* in 2011 (Table 3.3), when most pods formed during June. The overall level of damage across the UK was higher in 2011 than in other years, possibly indicating that earlier formation of pods led to greater levels of damage. This hypothesis is supported by further investigation of crops sown at different dates (Chapter 6).

Temperature conditions across England and Wales may not differ sufficiently to give large differences in mean percentage damage, although positive correlations were shown. However, it was seen that although *V. faba* was grown in Scotland (Figures 3.5 and 3.8), there were no recorded incidences of *B. rufimanus* damage to samples collected from Scotland (Appendix B, Table VI to X). Temperature was significantly below the rest of the UK for all years (Appendix B, Table XI) and it is likely that this was the key influencing factor preventing movement of *B. rufimanus* into Scotland. Damage levels were also lower in samples collected from the Scottish borders area of the UK, possibly for the same reason.

The analyses indicated that there may be an influence of temperature on activity of *B. rufimanus,* and consequent damage to grain. The weakness of the correlations may in part be due to unknown variables that influenced the dataset. A variable that was not accounted for in this study was the influence of insecticide applications. All samples were collected from commercial crops of *V. faba,* and it is likely that in England and Wales most crops had one or two insecticide applications each year to control *B. rufimanus.* Damage incidence by sample may have been influenced in part by the relative levels of experience in the management of *B. rufimanus* between different growers, and the different management strategies utilised by growers. Insecticides remained, in 2016, one of the least used classes of pesticide for all arable crops in the UK compared to fungicides, plant growth regulators, herbicides and seed treatments, and for field beans were used less by area than fungicides and herbicides (Garthwaite *et al.*, 2018). Pesticide Usage Statistics for 2016 showed that the regions with the greatest area of arable crops, and hence the greatest area of treated crops, were the Eastern region and the East Midlands. Field beans received on average two insecticide applications, with just under 30% of insecticide applications made to autumn sown field bean crops during May and almost 60% during June (Garthwaite *et al.,* 2017). Just over 30% of insecticide applications were made to spring sown field beans in May and around 45% in June. 33% of insecticide applications were made for bruchid beetle control and 5% for combined aphid and bruchid beetle control in 2016 (Garthwaite *et al.,* 2017). Lambda-cyhalothrin was the active substance most used with an average of 1.85 applications per crop where used, although there were no data to indicate geographical distribution of usage.

Long-term trends in the distribution and abundance of insect pests are difficult to record and document and it is possible that many more insect pests have responded to climate change, or are likely to do so, than are found in current literature (Battisti and Larsson, 2015). The data examined here indicated slight range expansion which was visible in 2011 and 2015, years in which *B. rufimanus* damage was recorded in more Northern and Western regions (Figures 3.6 and 3.8).

Other models are available to further test the effects of temperature and other meteorological influences, such as rainfall, on *B. rufimanus* damage. Significant effects could be determined using Generalised Linear Models (GLM), or Generalized Linear Mixed Models (GLMM), specifying random and fixed effects, although resources were not available to carry out these analyses. Although SAS® University Edition was suitable for moderate and smaller datasets, it was not powerful enough for the larger dataset used in this study. For statistical analyses, the R Programming Language (2017) would be appropriate. Generalized Linear Mixed Models are available using package 'lme4' (Bates *et al*., 2015). However, there may be limitations to this approach in effectively dealing with spatio-temporal correlations, and violations of independence. Other methods for modelling these data could be: A Generalized Additive Mixed Model that would include a temporal smoother to account for the fact that meteorological data may not be independent; a GLMM fit with Markov Chain Monte Carlo methods; spatio-temporal models for count data, for example INLA, an R package for fitting parametric spatial and spatio-temporal models (C. Davies, 2017, Personal Communication).

In summary, although there are some clear constraints present when using the dataset, the study has value to growers in assisting risk assessment and management. Further analysis may allow the development of more sophisticated predictions for activity of *B. rufimanus.*

Chapter 4: Conditions required to stimulate emergence of *B. rufimanus* from overwintering sites and commencement of oviposition

4.1 Introduction

There are several studies indicating the influence of both photoperiod and temperature on diapause and diapause termination in insects (Xiao *et al.,* 2006; Wang *et al.,* 2014). Photoperiod undergoes predictable change throughout the year, but climate systems are complex, and organisms may respond to changes in conditions or patterns over many years, including changes in extremes as well as mean values (Danks, 2006). Because many organisms are adapted to normal patterns of change which happen in different time frames, and their responses are complicated by other factors such as host-plant relationship and the presence of predators, trends for adaptation in insects may not be obvious. Generally, in predictable environmental or habitat conditions, insect life-cycles are simple and fixed (Danks, 2006). Obligate diapause, described as programmed arrested development, may end when environmental cues are present, such as photoperiod or temperature thresholds (Glitho *et al.,* 1996).

Changes in development and fecundity in relation to temperature are reported for the species *Acanthoscelides obtectus* Say. (Chrysomelidae: Bruchinae) (Soares *et al.,* 2015), and for other Chrysomelidae the importance of temperature, when other factors such as food availability, photoperiod and population density are constant, is the main determinant of species performance (Honek *et al.,* 2003). The duration of development, reproduction, fecundity, fertility and viability of offspring in many insect species are largely determined by temperature (Zhou *et al.,* 2010; Kutcherov, 2015; Hasan and Ansary, 2016).

Environmental factors influence the activity of *B. rufimanus*, and the effects of photoperiod and food source on the termination of reproductive diapause and commencement of oviposition are demonstrated by Tran and Huignard (1992) and Tran *et al.* (1993). Tran and Huignard (1992) and Medjdoub-Bensaad *et al.* (2007) described reproductive diapause in female *B. rufimanus* as reduction to germarium, and termination of reproductive diapause characterised by the presence of vitellogenesis in ovarioles and the presence of mature oocytes at the base of the ovarioles and lateral oviducts. They found that this was dependent on consumption of *V. faba* pollen. Reproductive development in male *B. rufimanus* was not dependent on the consumption of *V. faba* pollen. While there have been studies indicating the influence of temperature on activity of *B. rufimanus* (Franssen, 1955) and on the development of diapause in captive *B. rufimanus* (Tran *et al.* 1993), there is no work that describes the influence of temperature on emergence from natural overwintering sites or commencement of oviposition in field conditions in the UK. Evidence from data collected at PGRO in 2007 and 2008 indicated that oviposition commenced when temperature reached at least 20°C (A. Biddle, 2008, Personal Communication).

Better understanding the relationships between photoperiod, temperature and emergence of *B. rufimanus* from overwinter sites may allow further options for management of pest damage to be explored. Integrated pest management (IPM) strategies include the use of semiochemical-baited traps to monitor activity, or for mass capture (Loughlin, 2013). In this study traps were evaluated for their use to monitor pest emergence and efficacy in capturing large numbers of *B. rufimanus*. In modern agroecosystems, innovative IPM strategies are used to reduce the use of insecticides and other agrochemicals (Vinatier *et al.*, 2012). Semi-natural habitats such as hedgerows and field margins provide overwintering sites for many insects, and when trap systems are used in these habitats they may contribute to the management of pest populations.

Volatile secondary metabolites of plants can provide chemical signals that are specific to species, environmental conditions and plant organs. These can act as recognition cues for detection, colonization, quality assessment and location of mates by insects (Reinecke and Hilker, 2014; Pickett and Khan, 2016). Using gas chromatography-coupled electrophysiology with the insect antenna, it is possible to identify complex mixtures that mimic the natural host plant signal, although when delivered in artificial devices or traps, crops may be highly competitive when compared to traps (Pickett and Khan, 2016). Other difficulties may be presented when using plant semiochemicals in traps, particularly when mixtures are complex, and the release rate of each differs. This often leads to the use of separate release substrates. Bruce *et al.* (2011) investigated the attraction of *B. rufimanus* to *V. faba* plant volatiles using olfactometer bioassays and gas chromatography-electroantennography (GC-EAG). Electro-physiological recordings identified nine compounds from *V. faba* samples that elicited responses from *B. rufimanus*. These were identified as myrcene, (R)-limonene, (E)-ocimene, (R)-linalool, 4-allylanisole, cinnamyl alcohol, cinnamaldehyde, (E)-caryophyllene and α-humulene. Field testing of plant semiochemicals evaluated four treatments: Unbaited traps containing no semiochemicals; traps containing a mixture of three plant semiochemicals ((R)-linalool, cinnamylalcohol and cinnamaldehyde); traps containing the nine plant semiochemicals identified above; and traps containing the nine plant semiochemicals with an insect pheromone, 1-undecene. Bruce *et al.* (2011) found that there was no statistically significant difference between the three baited traps, and that the simpler mixture of three plant semiochemicals was sufficient for field monitoring.

Changes in the seasonal timing of biological or phenological events, such as breeding and migration of organisms, have occurred due to climate warming, with many spring and summer life-cycle events, for a wide range of taxa, occurring earlier in the year than historically (Knell and Thackeray, 2016). Estimates of mean number of days change in spring phenology for all taxa studied, based on temperature warming, are between 2.3 and 5.1 days earlier in a decade, or 0.39 days in a year (Root *et al.,* 2003; Thackeray *et al.,* 2010). Timing of emergence of organisms from diapause may impact on the survival of adults, particularly when food resources are not available, or climate conditions are sub-optimal. There is evidence that female *B. rufimanus* requires *V. faba* pollen to end reproductive diapause (Tran *et al.,* 1992) and when spring emergence from overwinter sites occurs during mid-April, *V. faba* flowers may not be present. There are reports that temperature is required to reach between 17 and 20°C for adults to colonise crops (Biddle and Cattlin, 2007; Roubinet, 2016), although there are no published studies examining the migration of *B. rufimanus* into crops. The lack of evidence to support the hypothesis that temperature and photoperiod are determinants of migration of *B. rufimanus* into *V. faba* crops led to this investigation to evaluate the effects of both factors on adult migration in spring. Identification of the conditions required to stimulate adult emergence from overwinter sites may lead to an improved understanding of the link between emergence and crop damage, or opportunities to manage *B. rufimanus* populations prior to movement into crops. Chapter 3 discussed the relationship between temperature and crop damage for the UK, attempting to provide evidence that crop damage by *B. rufimanus* could be predicted by temperature.

In this study, monitoring of adult activity and crop growth stage was undertaken at several sites in East Anglia between 2009 and 2014 to determine the primary factors influencing emergence of *B. rufimanus* from overwintering sites. Comparisons were made between the number of adults recorded in traps and mean daily temperature and photoperiod, determined by day length inclusive of civil twilight. Oviposition and *V. faba* crop growth stage were recorded in 2009 to determine the primary factors influencing the commencement of oviposition.

4.2 Methods

4.2.1 *Monitoring of adult B. rufimanus on emergence from overwintering sites*

Monitoring was undertaken at commercial field bean (*V. faba minor*) sites in East Anglia between 2009 and 2014 (Table 4.1) using attractant traps containing plant semiochemicals that have been proven to stimulate responses in *B. rufimanus* (Bruce *et al.*, 2011). Monitoring traps were of the 'cone' type, similar to those used to monitor boll weevils in cotton in the USA and South America and were mounted on one metre poles (Plate 4.1). The attractant contained plant semiochemicals supplied by Rothamsted Research and was a mixture of cinnamyl alcohol, trans-cinnamylaldehyde and linalool released from pieces of cellulose sponge contained in polythene sachets. These semiochemicals are released from *V. faba* flowers at a ratio of 1:2:44. Following preliminary testing in 2008, the semiochemical attractants released at the ratio 1:2:44 were found to have the highest success in attracting *B. rufimanus* when compared to unbaited cone traps or traps baited with lures containing the three component semiochemicals at ratios of 1:1:1 and 1:10:10. Attractants used in all monitoring experiments for this study contained the plant semiochemicals released at the ratio of 1:2:44.

B. rufimanus is attracted to the semiochemical lure, flying into the trap via the base and moving towards the top of the trap where they enter a small bulb from which they cannot escape. Monitoring sites were selected that had a history of *V. faba* cropping and high reported populations of *B. rufimanus*. Traps were located in the field margins of commercial spring sown *V. faba* crops and on field margins or undisturbed grassy field corners in fields in which *V. faba* was grown in the previous season, placed at a height of 0.8 metres and at 20 metre intervals to intercept adult *B. rufimanus* as they emerged from overwintering sites. At Crowland in 2009 and Tilney St. Lawrence in 2014 traps were moved from overwintering sites to crop margins in May, to continue monitoring. All traps were monitored regularly until first insects were recorded, followed by recording at intervals of between 3 and 7 days. Once recorded, adult *B. rufimanus* were removed from the sites at each observation to avoid repeated catch and observation. Met Office datasets for mean and maximum daily temperature were downloaded from the data server of the Centre for

Environmental Data Analysis (Met Office, 2017). The datasets contain daily observations of temperature interpolated to a uniform 5 kilometre grid resolution covering the period 1960 to 2016. The input station data originate from the Met Office Integrated Data Archive System and provide observations relating to periods 0900 hours to 0900 hours (Met Office, 2017). Photoperiod, including civil twilight, was calculated and recorded from the website www.timeanddate.com.

Table 4.1: Locations used to monitor the emergence of *B. rufimanus* from overwintering sites using modified boll weevil traps containing plant semiochemical attractants. Traps were placed in field margins and uncropped areas surrounding *V. faba* crops in 2009 to 2014.

Site name	Site grid reference	Number of traps at each site and description of habitat	Date of trap placement at each site
Bourne, Lincolnshire	TF087213	4 on margin of *V. faba* crop	14 May 2009
Crowland, Lincolnshire	TF268138 TF270135	4 on grassy field corner area of previous *V. faba* crop and moved to margin of *V. faba* crop	31 March 2009 14 May 2009
Crowland, Lincolnshire	TF270135	4 on margin of previous *V. faba* crop	16 March 2010
Tilney St. Lawrence, Norfolk	TF566144	12 on margin of *V. faba* crop	04 March 2011
Tilney St. Lawrence, Norfolk	TF574146	12 on margin of *V. faba* crop	15 March 2012
Tilney St. Lawrence, Norfolk	TF571147	12 on margin of *V. faba* crop	03 March 2013
Tilney St. Lawrence, Norfolk	TF571147 TF556139	4 on margin of previous *V. faba* crop and 8 placed on margin of *V. faba* crop	19 March 2014 13 May 2014

Plate 4.1: Modified boll weevil monitoring traps containing plant semiochemical lures to attract *B. rufimanus*, placed along the field margins of commercial spring sown *V. faba* crops.

Data were controlled for number of traps using mean number of *B. rufimanus* per trap recorded at each observation. The first analyses used all of the trap data for the duration of monitoring. Spearman's rank correlation coefficient was calculated for all data recorded, to provide a preliminary indication of the strength of the relationship between temperature and number of adults recorded in traps, and photoperiod and number of adults recorded in traps. Regression analyses were conducted following correlation. The number of adult *B. rufimanus* recorded in traps was analysed against temperature and photoperiod to determine whether the effectiveness of the traps was influenced by these factors. The second set of analyses used observations at the time of first emergence to determine whether there was an effect on emergence of *B. rufimanus* from overwinter sites. Data from Tilney St. Lawrence were analysed separately to determine whether site influenced emergence behaviour or trap effectiveness.

4.2.2 *Oviposition*

Oviposition by *B. rufimanus* was recorded at a commercial broad bean (*V. faba major*) site at East Raynham in Norfolk (Grid reference TF892251) in 2009. Two crop areas, measuring approximately two hectares each, were sown by the grower on two dates, the first on 18 March 2009 and the second on 31 March 2009. The areas had no insecticides applied to control *B. rufimanus*. Monitoring of oviposition began from the development of first pods and continued at approximately 14-day intervals. It is reported that a period of between 11 and 21 days is required for larvae to hatch and bore through the pod wall (Roubinet, 2016). This depends on temperature, although no data on degree-days is available. Newly laid eggs were recorded at each pod bearing node on ten plants selected at random within each area. The age of the eggs was determined by colour and transparency, young eggs appearing pale cream in colour compared to older eggs which appear slightly yellow. More recently laid eggs appear more opaque than older eggs due to the presence of first instar larvae within the eggs, and older eggs were reduced to the chorion only (Medjdoub *et al.,* 2007) (Plates 4.2 and 4.3). The duration of oviposition was recorded.

Plate 4.2: Recently laid egg of *B. rufimanus* on *V. faba* pod. Eggs are pale cream to white colour and opaque due to presence of first instar larva.

Plate 4.3: Hatched eggs of *B. rufimanus* on *V. faba* pod. Eggs are yellow and translucent, reduced to the chorion only.

4.3 Results

4.3.1 *Monitoring of adult B. rufimanus on emergence from overwintering sites*

B. rufimanus adults were first recorded in the monitoring traps placed at Crowland in 2009 on 20 April (Figure 4.1). On this date a single adult was recorded, and mean daily temperature was 9.59°C. Photoperiod including civil twilight on 20 April 2009 was 15 hours and 31 minutes. Only two adult *B. rufimanus* were recorded during trap observations between 23 April and 28 May 2009 at Crowland, and during this period highest mean daily temperature occurred on 28 may, at 16.66°C. Despite high average maximum daily temperature between 28 May and 03 June of 27.06°C, average mean daily temperature was 14.19°C and only 2 adults were recorded on 03 June. *V. faba* flowering started on 28 May and ended on 22 June 2009 at Crowland, and it is possible that competition from the flowering crop reduced effectiveness of traps.

First adult *B. rufimanus* were recorded in the monitoring traps located on the field margins of the *V. faba* crops at Bourne on 28 May 2009. During the seven-day period preceding this, mean daily temperature ranged from 11.85°C to 16.44°C (Figure 4.2). Photoperiod on 28 May 2009 was 17 hours and 57 minutes. *V. faba* flowering started on 03 June and ended on 25 June 2009 at Bourne, and few adults were recorded in traps during this period.

Following the end of flowering at both sites, large numbers of adult *B. rufimanus* were recorded in traps, indicating that the competition with flowering crops was no longer influencing trap catches.

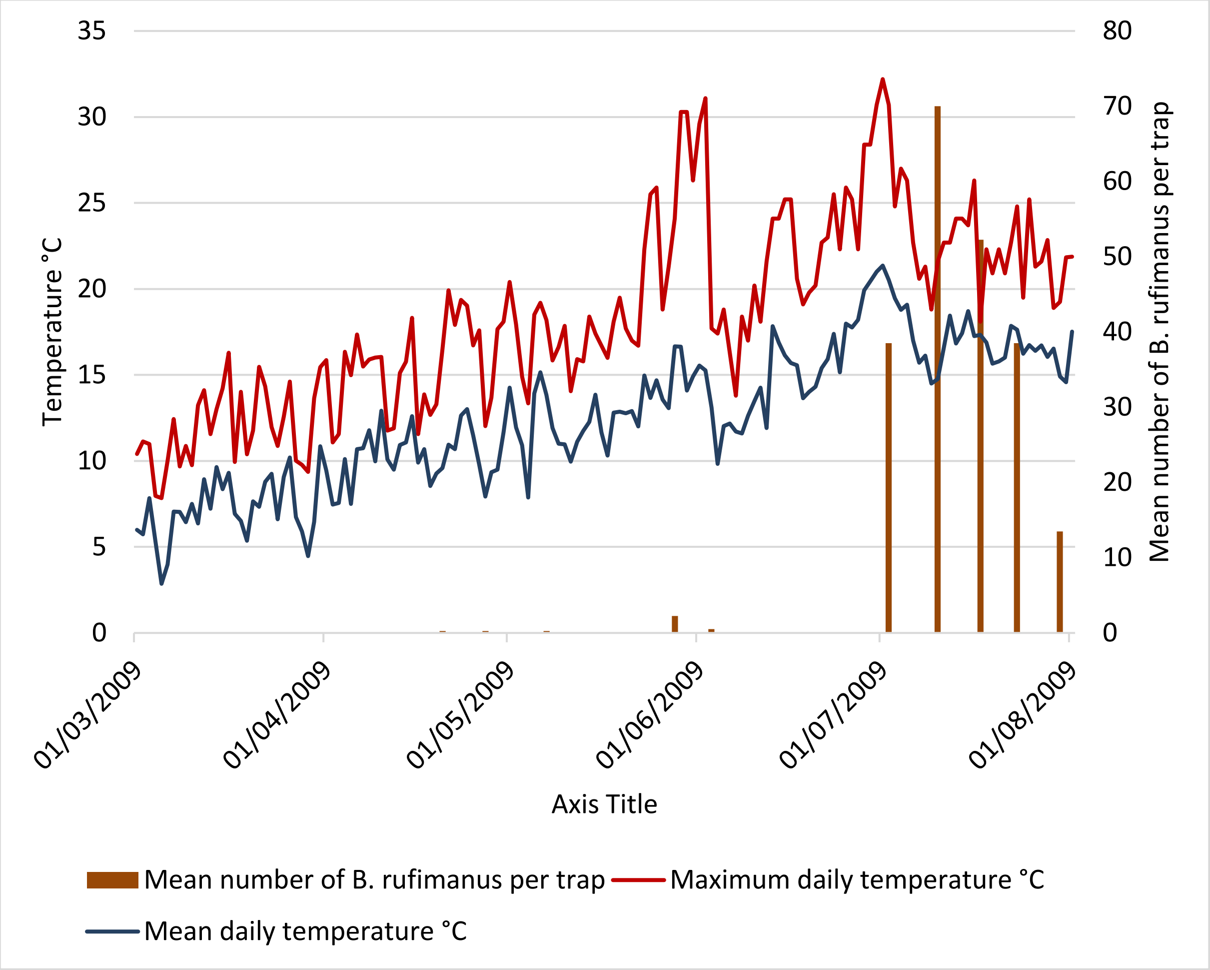


Figure 4.1: Mean number of adult *B. rufimanus* recorded in attractant monitoring traps located at Crowland in 2009, with mean and maximum daily temperature.

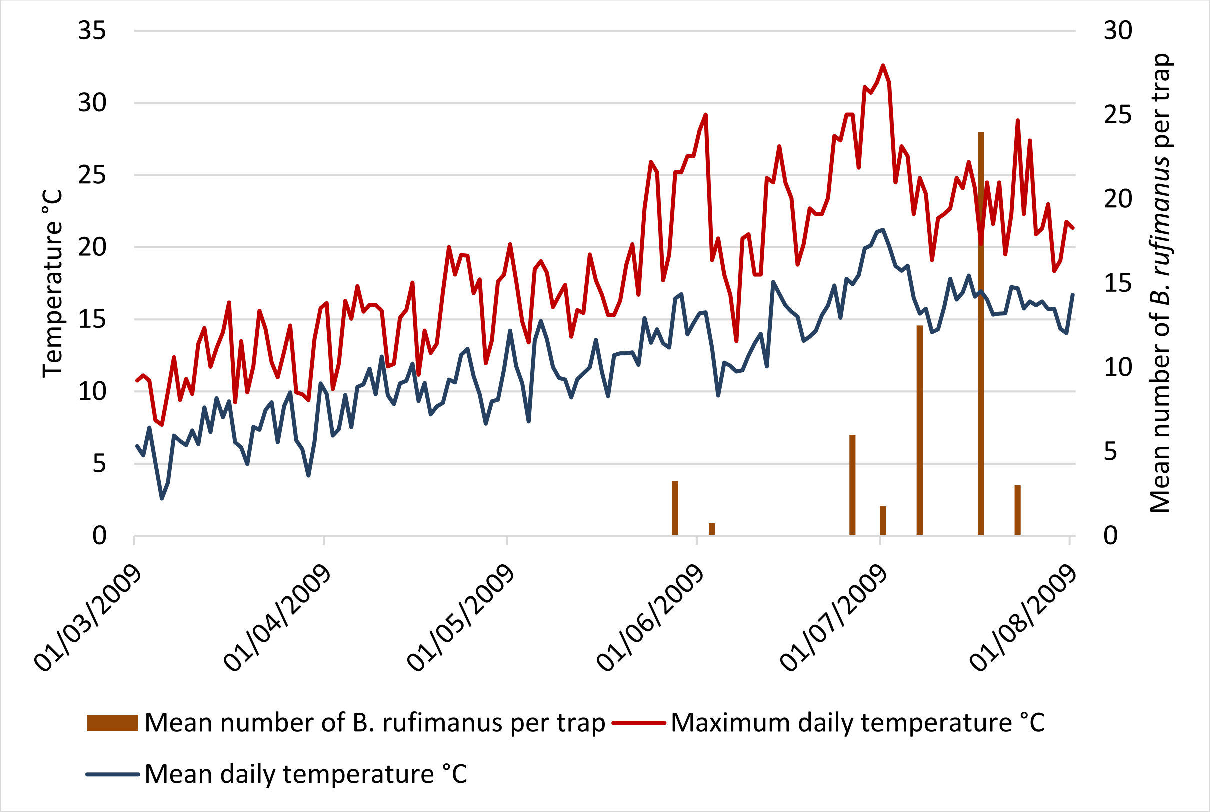


Figure 4.2: Mean number of adult *B. rufimanus* recorded in attractant monitoring traps located at Bourne in 2009, with mean and maximum daily temperature.

Adult *B. rufimanus* were first recorded in monitoring traps located at Crowland on 11 May 2010 (Figure 4.3), none having been recorded at regular observations during the period from 19 March. Although temperature reached 21.6°C on 27 April and 21.7°C on 28 April, no adults were recorded in traps on 30 April. At this date photoperiod was 16 hours and 13 minutes and by 11 May had increased to 16 hours and 58 minutes. The highest mean daily temperature reached in the seven days prior to 11 May 2010 was 10.54°C. Only a single adult was recorded on 11 May and four were recorded on 18 May when mean daily temperature was 10.77°C and photoperiod 17 hours and 24 minutes. None of the surrounding *V. faba* crops was flowering by 11 may.

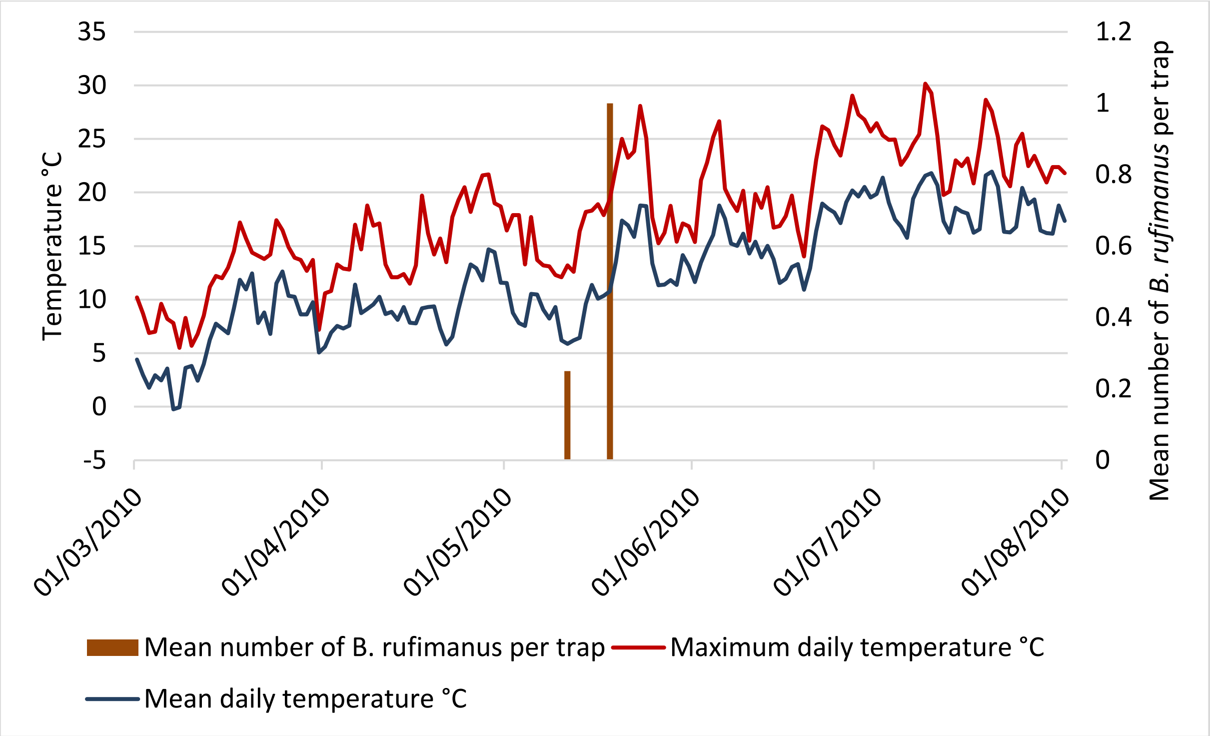


Figure 4.3: Mean number of adult *B. rufimanus* recorded in attractant monitoring traps located in the margin of a field containing *V. faba* in the previous year at Crowland in 2010, with mean and maximum daily temperature.

Three adult *B. rufimanus* were recorded in monitoring traps located in field margins of the *V. faba* crop at Tilney St. Lawrence on 14 April 2011 (Figure 4.4). Mean daily temperature ranged between 6.16°C and 12.03°C in the seven-day period between 08 and 14 April (Figure 4.4). Photoperiod on 14 April was 15 hours and four minutes. The number of adult *B. rufimanus* recorded in traps increased significantly on 27 April following a period of higher temperature between 20 and 23 April, when the mean daily temperature at Tilney St.

Lawrence reached a peak of 17.0°C, and 86 adults were recorded in the traps. Photoperiod on 27 April was 16 hours. At this time surrounding *V. faba* crops were not flowering and flowering occurred from 26 May until the end of June, a period during which fewer adult *B. rufimanus* were recorded in traps. Following the flowering period, large numbers of adult *B. rufimanus* were recorded in traps, again due to lack of competition with the flowering crop.

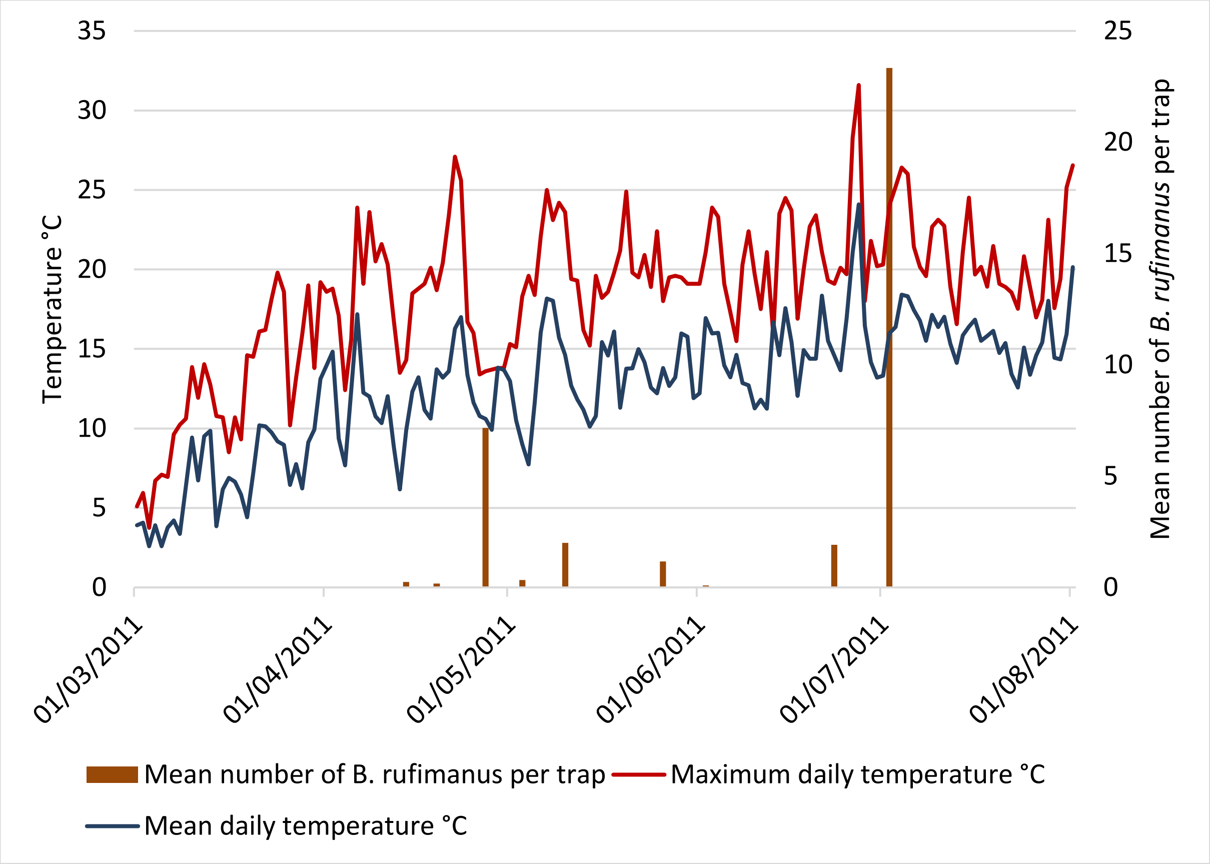


Figure 4.4: Mean number of adult *B. rufimanus* recorded in attractant traps located at Tilney St. Lawrence in 2011, with mean and maximum daily temperature.

Data from monitoring traps located in the field margins of the *V. faba* crop at Tilney St. Lawrence in 2012 showed a clearer response to temperature by adult *B. rufimanus* (Figure 4.5) than in previous years. Two adults were recorded on 13 May 2012 when average mean daily temperature was 11.64°C in the seven-day period prior to this date. Photoperiod on 13 May was 17 hours and seven minutes. Four adults were recorded on 24 May 2012 following a seven-day period when average mean daily temperature was 12.95°C, and on 24 May mean daily temperature was 16.34°C. Following a period of seven days when mean daily temperature ranged from 15.13°C to 17.24°C between 25 May and 31 May, 46 adults were recorded in monitoring traps. Flowering began on 1 June and fewer adults were recorded in traps after this date.

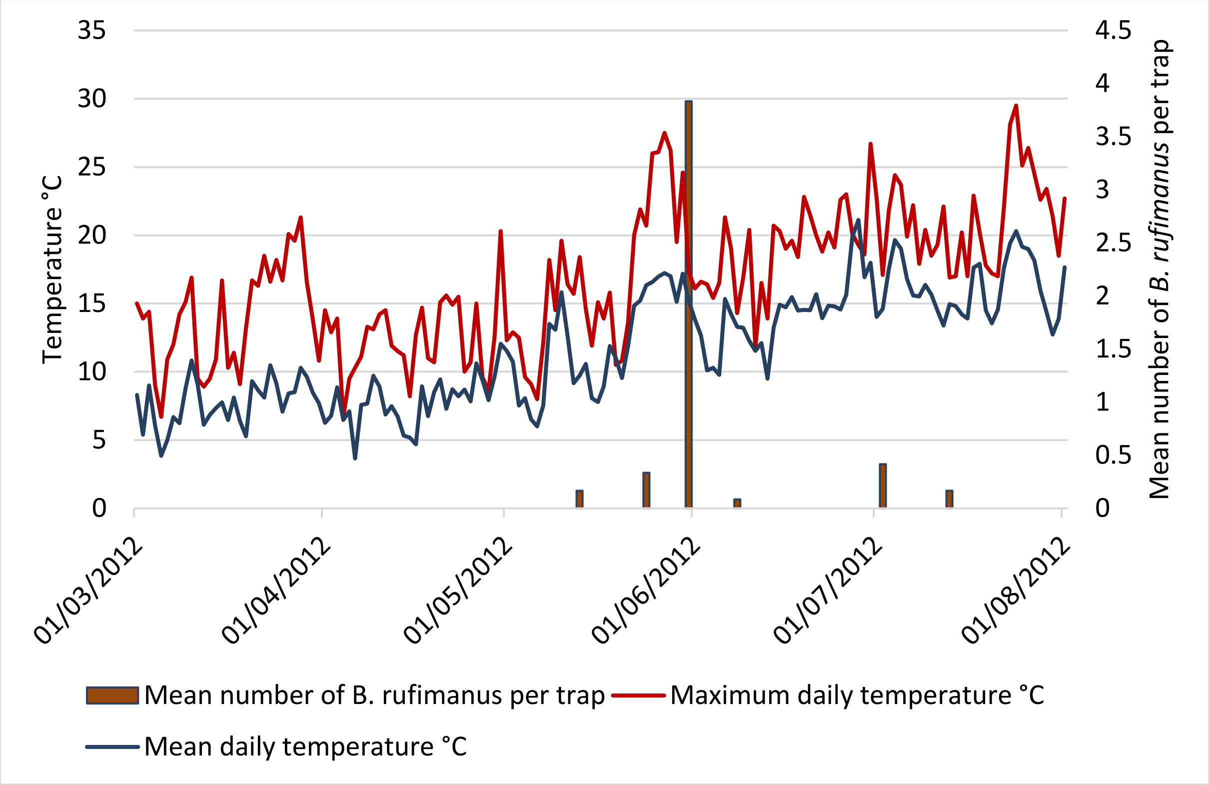


Figure 4.5: Mean number of adult *B. rufimanus* recorded in attractant monitoring traps located at Tilney St. Lawrence in 2012, with mean and maximum daily temperature.

The response of adult *B. rufimanus* to temperature in 2013 was less clear, and no adults were recorded in traps on 10 May, despite average mean daily temperature in the seven-day period prior to this being 12.97°C. Average mean temperature was lower prior to the first adult recorded in monitoring traps on 21 May, at 10.49°C in the seven days preceding the first record of *B. rufimanus* in monitoring traps (Figure 4.6). Photoperiod on 21 May 2013 was 17 hours and 34 minutes. On this date a single adult was recorded, as on 03 June 2013. Mean daily temperature during the period 21 May to 03 June was 10.81°C, and adult emergence from overwintering sites was low. Flowering in the *V. faba* crop began on 11 June and no further adults were recorded in traps until flowering ended in mid-July (Figure 4.6).

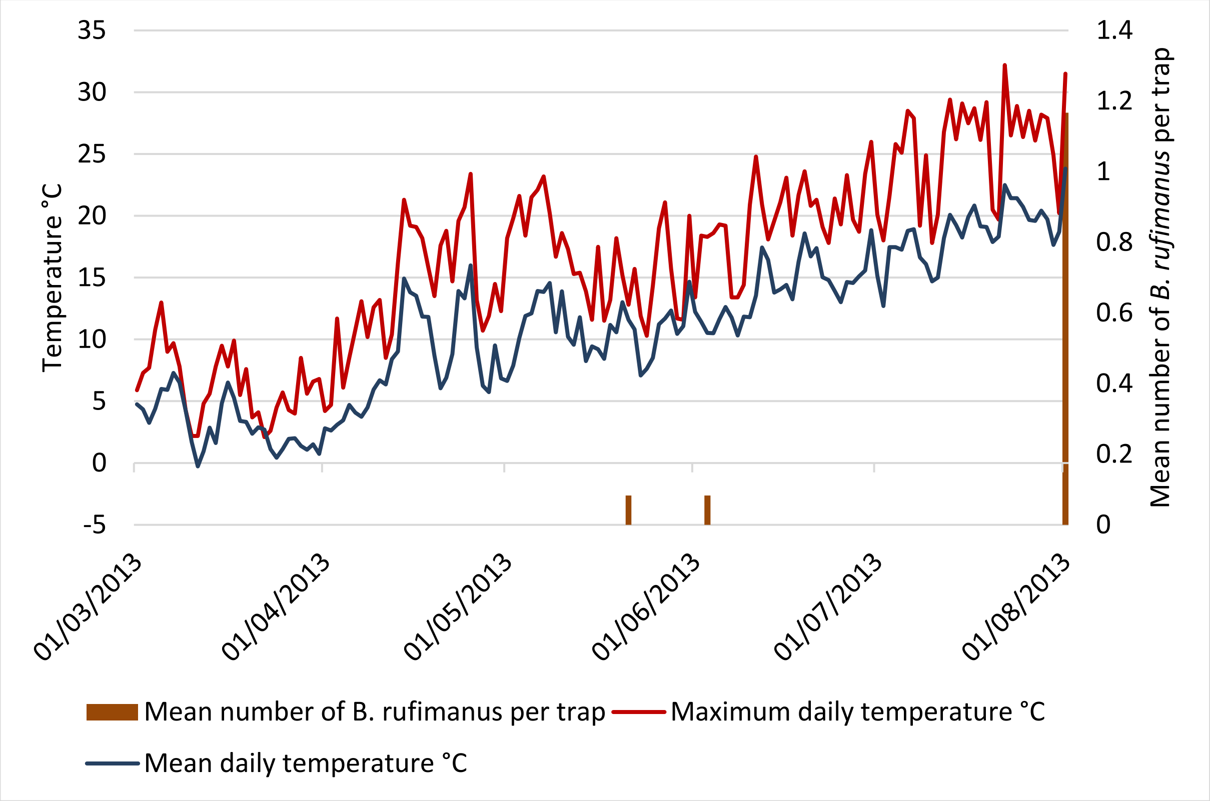


Figure 4.6: Mean number of adult *B. rufimanus* recorded in attractant monitoring traps located at Tilney St. Lawrence in 2013, with mean and maximum daily temperature.

Data from monitoring traps located at Tilney St. Lawrence in 2014 showed that no *B. rufimanus* emerged between before 06 May, when mean daily temperature reached 14.59°C (Figure 4.7). Photoperiod on 6 May was 16 hours and 38 minutes. Twenty-one adults were recorded on 06 May.

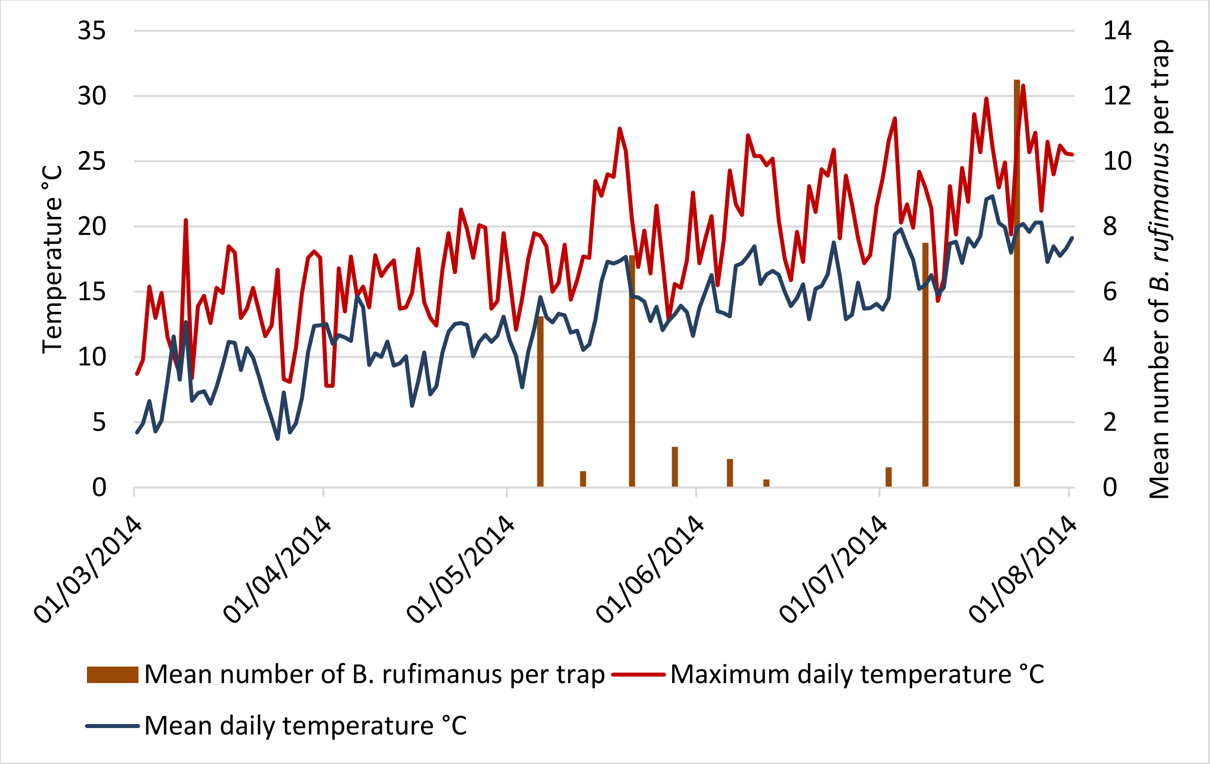


Figure 4.7: Mean number of adult *B. rufimanus* recorded in attractant monitoring traps located at Tilney St. Lawrence in 2014, with mean and maximum daily temperature.

Data collected from monitoring traps located on the field margins surrounding the *V. faba* crop at Tilney St. Lawrence in 2014 appeared to indicate that prolonged periods of higher temperature led to greater numbers of *B. rufimanus* recorded in traps (Figure 4.7). Adults continued to be recorded in monitoring traps in low numbers during the *V. faba* flowering period, which started between 29 May and 01 June and fewer adult *B. rufimanus* were recorded in traps between 29 May and 5 June as the flowering crop competed with the traps.

At all sites, the traps attracted many more adult *B. rufimanus* towards the end of the monitoring period, as crops ended the flowering stage and were no longer out-competing the traps.

Data from trap observations were checked for normality and homogeneity using the Anderson-Darling and Levenes tests, respectively. Spearman correlations were conducted to investigate the relationship between temperature and observation of adult *B. rufimanus* in traps, including the influence of temperature on emergence of *B. rufimanus* from overwinter sites, and to investigate the effect of photoperiod on trap catch and emergence (Tables 4.2 and 4.3). Various time frames were examined leading up to and extending beyond the time of first trap recordings, using mean daily temperature. The results of these analyses showed that mean daily temperature showed low to moderate, and statistically significant correlation to numbers of *B. rufimanus* recorded in traps for different time frames and these are shown in Tables 4.2 and 4.3.

Table 4.2: Spearman's correlation for all trap records, 2009-2014, for average mean daily temperature during varying periods prior to the day of observation, correlated with mean number of adult *B. rufimanus* recorded in traps at each observation, including descriptive statistics for mean number of adult *B. rufimanus,* with Spearman correlation of photoperiod and mean number of adult *B. rufimanus* per observation. Total number of observations is 74.

Variable	Number of observations	Mean	Std. Dev.	Median	Min	Max
Mean number of adult *B. rufimanus* per trap	74	4.694	12.197	0.25	0	70

Correlated with mean number of adult *B. rufimanus* recorded in traps

All temp. measured in °C

Variable	**Sample correlation**	**P value**	**Fishers Z**	**Bias adj**	**Correlation estimate**	**95% confidence limits**		**P value**
Mean temp. on day of catch	0.40827	0.0003	0.43353	0.00280	0.40594	0.195578	0.580582	0.0003
Average mean daily temperature during 2 days prior to obs.	0.43180	0.0001	0.46210	0.00296	0.42939	0.222743	0.599105	<.0001
Average mean daily temperature during 7 days prior to obs.	0.47778	<.0001	0.52011	0.00327	0.47525	0.276816	0.634815	<.0001
Average mean daily temperature during 14 days prior to obs.	0.49839	<.0001	0.54717	0.00341	0.49582	0.3014582	0.650612	<.0001
Average mean daily temperature during 28 days prior to obs.	0.50956	<.0001	0.56214	0.00349	0.50697	0.314959	0.659117	<.0001
Photoperiod on day of obs.	0.24554	0.0350	0.25066	0.00168	0.24396	0.016371	0.447510	0.0347

Table 4.3: Spearman's correlation for trap records, 2009-2014, for mean daily temperature during varying periods prior to the day of observation, correlated with the number of adult *B. rufimanus* recorded in traps at the first recorded catch, with descriptive statistics for the observed number of adult *B. rufimanus* and Spearman correlation for photoperiod and number of adult *B. rufimanus* per observation. Total number of observations is 21.

Variable	Number of observations	Mean	Std. Dev.	Median	Min	Max
Mean number of adult *B. rufimanus* per trap	21	0.452	1.305	0	0	5.25

	Correlated with mean number of adult *B. rufimanus* recorded in traps					All temp. measured in °C		
Variable	**Sample correlation**	**P value**	**Fishers Z**	**Bias adj**	**Correlation estimate**	**95% confidence limits**		**P value**
Mean temp. on day of obs.	0.08138	0.7258	0.08156	0.00203	0.07936	-0.364827	0.494118	0.7293
Average mean daily temperature during 2 days prior to obs.	0.11163	0.6158	0.11678	0.00291	0.11339	-0.334680	0.519640	0.6203
Average mean daily temperature during 7 days prior to obs.	0.28367	0.2127	0.29166	0.00709	0.27713	-0.175557	0.633081	0.2159
Average mean daily temperature during 14 days prior to obs.	0.62004	0.0027	0.72506	0.01550	0.61040	0.242656	0.824762	0.0021
Average mean daily temperature during 28 days prior to obs.	0.50145	0.0206	0.55125	0.01254	0.49201	0.076592	0.761879	0.0193
Photoperiod on day of obs.	0.58516	0.0053	0.670274	0.01463	0.57546	0.191289	0.806736	0.0045

Numbers of adult *B. rufimanus* recorded in traps were moderately correlated with mean daily temperature leading up to the date of observation, and correlations were highly significant (Table 4.2). The correlation between photoperiod on the day of observation and number of *B. rufimanus* recorded at each observation was weak and significant (Table 4.2). These data do not indicate a causative relationship between temperature and observation of adults in traps, nor do they indicate a relationship between temperature and emergence as all trap observations were analysed. They may indicate how well the traps performed as temperature increased.

To consider the relationship between temperature and emergence, the correlations for observations leading up to first record of adults were examined (Table 4.3). There was a strong correlation between mean daily temperature during the 14 days prior to observation and observation of adult *B. rufimanus* in traps (Table 4.3). The correlation between photoperiod on the day of observation and number of *B. rufimanus* recorded at each observation was moderate and significant (Table 4.3).

Regression analyses were undertaken to further examine some of the correlations found within the data, and to evaluate whether either temperature or photoperiod influenced the number of adult *B. rufimanus* observed in traps. These were conducted for all sites and all data (Table 4.4), with additional analyses for Tilney St. Lawrence alone (Table 4.5), and for data from all sites up to the time of first adults recorded in traps (Table 4.6). Data from Tilney St. Lawrence were analysed separately to determine whether site-specific factors influenced success of traps (Table 4.5).

Table 4.4: Regression analyses for mean daily temperature during different periods leading up to the day of observation and photoperiod on the day of observation, with mean number of *B. rufimanus* per trap at each observation for all observations at all sites 2009 to 2014.

Number of observations 74
Y variable = mean number of B. rufimanus per trap

X Variable	R Square	Adjusted R Square	SE	Coeff.	SE Coeff.	t Stat	P-value
Mean temperature on day of observation	0.0834	0.0707	11.7580	0.9657	0.3773	2.5592	0.0126
(Intercept)				-8.7060	5.4115	-1.6088	0.1120
Mean daily temperature during 2 days prior to observation	0.0947	0.0821	11.6850	1.0652	0.3881	2.7447	0.0076
(Intercept)				-9.9298	5.4985	-1.8059	0.0751
Mean daily temperature during 7 days prior to observation	0.1471	0.1352	11.3422	1.3936	0.3955	3.5234	0.0007
(Intercept)				-14.4491	5.5909	-2.5844	0.0118
Mean daily temperature during 14 days prior to observation	0.1862	0.1749	11.0787	1.6361	0.4031	4.0593	0.0001
(Intercept)				-17.0526	5.5099	-3.0949	0.0028
Mean daily temperature during 28 days prior to observation	0.2060	0.1950	10.9431	1.7642	0.4082	4.3225	<.0001
(Intercept)				-17.635	5.3202	-3.3148	0.0014
Photoperiod on day of observation	0.0375	0.0241	12.0487	1.6451	0.9823	1.6748	0.0983
(Intercept)				-23.5256	16.9083	-1.3914	0.1684

Table 4.5: Regression analyses for mean daily temperature during different periods leading up to the day of observation and photoperiod on the day of observation, with mean number of *B. rufimanus* per trap at each observation for all observations at Tilney St. Lawrence 2011 to 2014.

Number of observations 38

Y variable = mean number of B. rufimanus per trap

X Variable	R Square	Adjusted R Square	SE	Coeff	SE Coeff	t Stat	P-value
Mean temperature on day of observation	0.0810	0.0555	4.3833	0.3820	0.2145	1.7812	0.0833
(Intercept)				-3.1250	3.0014	-1.0411	0.3048
Mean daily temperature during 2 days prior to observation	0.0689	0.0430	4.4121	0.3913	0.2397	1.6322	0.1114
(Intercept)				-3.1943	3.3032	-0.9670	0.3400
Mean daily temperature during 7 days prior to observation	0.1653	0.1421	4.1775	0.6184	0.2316	2.6696	0.0113
(Intercept)				-6.43400	3.2564	-1.9758	0.0559
Mean daily temperature during 14 days prior to observation	0.1229	0.0985	4.2823	0.5870	0.2614	2.2456	0.0310
(Intercept)				-5.7619	3.5558	-1.620	0.1139
Mean daily temperature during 28 days prior to observation	0.0828	0.0573	4.3790	0.4999	0.2773	1.8026	0.0798
(Intercept)				-4.3015	3.6049	-1.1933	0.2406
Photoperiod on day of observation	0.0162	-0.0111	4.5351	0.4273	0.5544	0.7708	0.4459
(Intercept)				-5.3368	9.6364	-0.5538	0.5831

Table 4.6: Regression analyses for mean daily temperature during different periods leading up to the day of observation and photoperiod on the day of observation, compared to mean number of *B. rufimanus* per trap at each observation at the date of first recorded adults at all sites 2009 to 2014.

Number of observations 21
Y Variable = mean number of *B. rufimanus* per trap

X Variable	R Square	Adjusted R Square	SE	Coeff.	SE Coeff.	t Stat	P-value
Mean temperature on day of observation	0.2550	0.2158	1.1555	0.2314	0.0907	2.5500	0.0196
(Intercept)				-2.0186	1.0013	-2.0160	0.0582
Mean daily temperature during 2 days prior to observation	0.1988	0.1566	1.1983	0.2385	0.1098	2.1712	0.0428
(Intercept)				-2.0133	1.1653	-1.7277	0.1003
Mean daily temperature during 7 days prior to observation	0.1382	0.0928	1.2428	0.2604	0.1492	1.7454	0.0971
(Intercept)				-2.1964	1.5417	-1.4247	0.1705
Mean daily temperature during 14 days prior to observation	0.2256	0.1848	1.1781	0.3747	0.1593	2.3525	0.0296
(Intercept)				-3.1917	1.5702	-2.0329	0.0563
Mean daily temperature during 28 days prior to observation	0.1828	0.1398	1.2102	0.3371	0.1635	2.0615	0.0532
(Intercept)				-2.6196	1.5134	-1.7309	0.0997
Photoperiod on day of observation	0.1013	0.0540	1.2691	0.2596	0.1775	1.4631	0.1598
(Intercept)				-3.6181	2.7959	-1.2941	0.2112

Regression analysis showed weak positive relationships between temperature prior to observation and the number of *B. rufimanus* recorded in traps (Table 4.4). The relationship was slightly stronger when a longer time period was considered and 20.6% of variation in number of *B. rufimanus* recorded was explained by mean daily temperature for 28 days prior to observation. As temperature increased, so did the number of adults recorded in traps, although the relationship was weak. All regression analyses for mean daily temperature were statistically significant (Table 4.4). These results may indicate that higher temperature over a period of time caused traps to be more effective, although this is very inconclusive from the weak relationships found. When all data were considered, including records of catches after first emergence, photoperiod had no effect on trap effectiveness.

Analyses conducted on data recorded at Tilney St. Lawrence showed very weak relationships between mean daily temperature and trap records and these were not statistically significant in most cases, indicating that there were no site-specific factors influencing success of traps (Table 4.5).

When data were analysed for recorded number of adult *B. rufimanus* at all sites at the point of first catch, the relationship between mean daily temperature and number of adult *B. rufimanus* recorded in traps was weak (Table 4.6). The relationship between photoperiod and number of adult *B. rufimanus* recorded in traps was weak and not significant.

At most the data showed that the traps may have been more effective as temperature increased, but it is not possible to conclude that recorded number of *B. rufimanus* in traps indicated whether temperature or photoperiod influenced emergence.

No adult *B. rufimanus* were recorded in monitoring traps when photoperiod was less than 15 hours on the day of observation (12 to 13 April in all years) (Figure 4.8). No adults were recorded in traps when mean daily temperature during the 28-day period prior to observation was below 9°C (Figure 4.9).

Figure 4.10 shows all observations for all sites in all years plotted against photoperiod and mean daily temperature during the 28 days prior to the observation. As mean daily temperature and photoperiod increased adults were more likely to be recorded in traps, and no adults were recorded below 15 hours photoperiod or 9°C mean daily temperature. There were also occasions when no adults were recorded in traps at high mean daily temperature and photoperiod prior to the observation, possibly as a result of competition from the flowering crop.

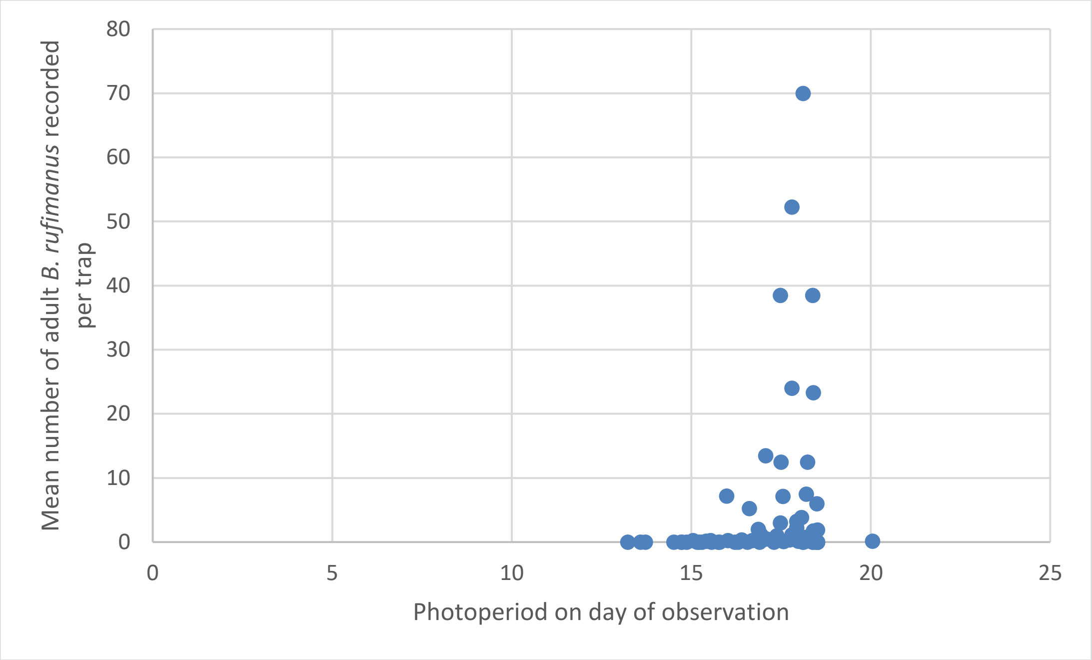


Figure 4.8: Mean number of adult *B. rufimanus* recorded in attractant monitoring traps at overwintering sites between 2009 and 2014 at Crowland, Bourne and Tilney St. Lawrence compared to photoperiod on the day of trap observation.

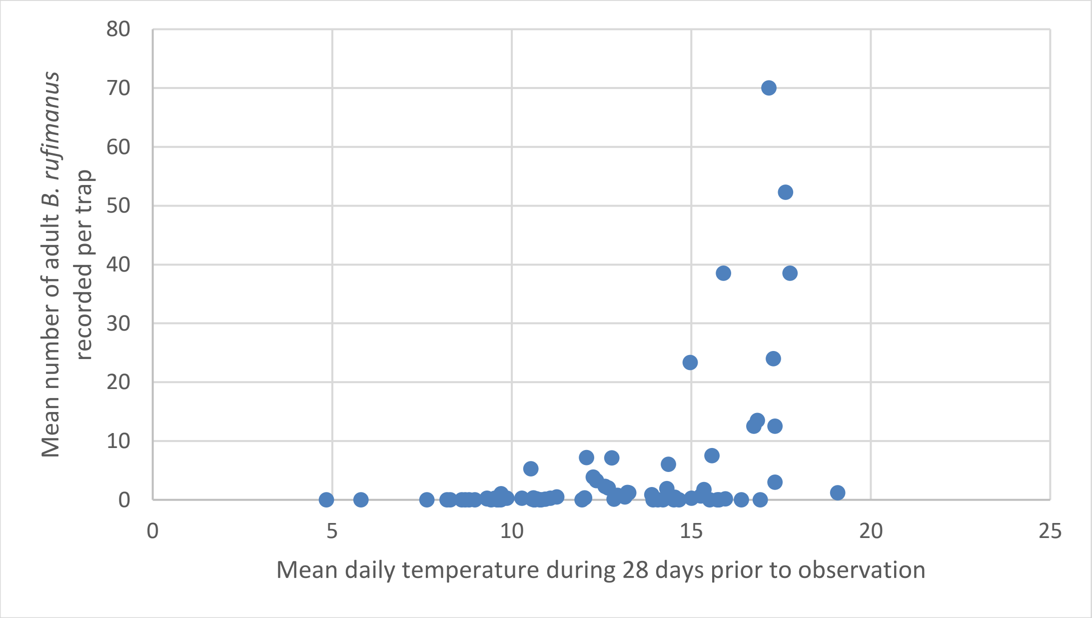


Figure 4.9: Mean number of adult *B. rufimanus* recorded in attractant monitoring traps at overwintering sites between 2009 and 2014 at Crowland, Bourne and Tilney St. Lawrence compared to mean daily temperature during the 28 days prior to observation.

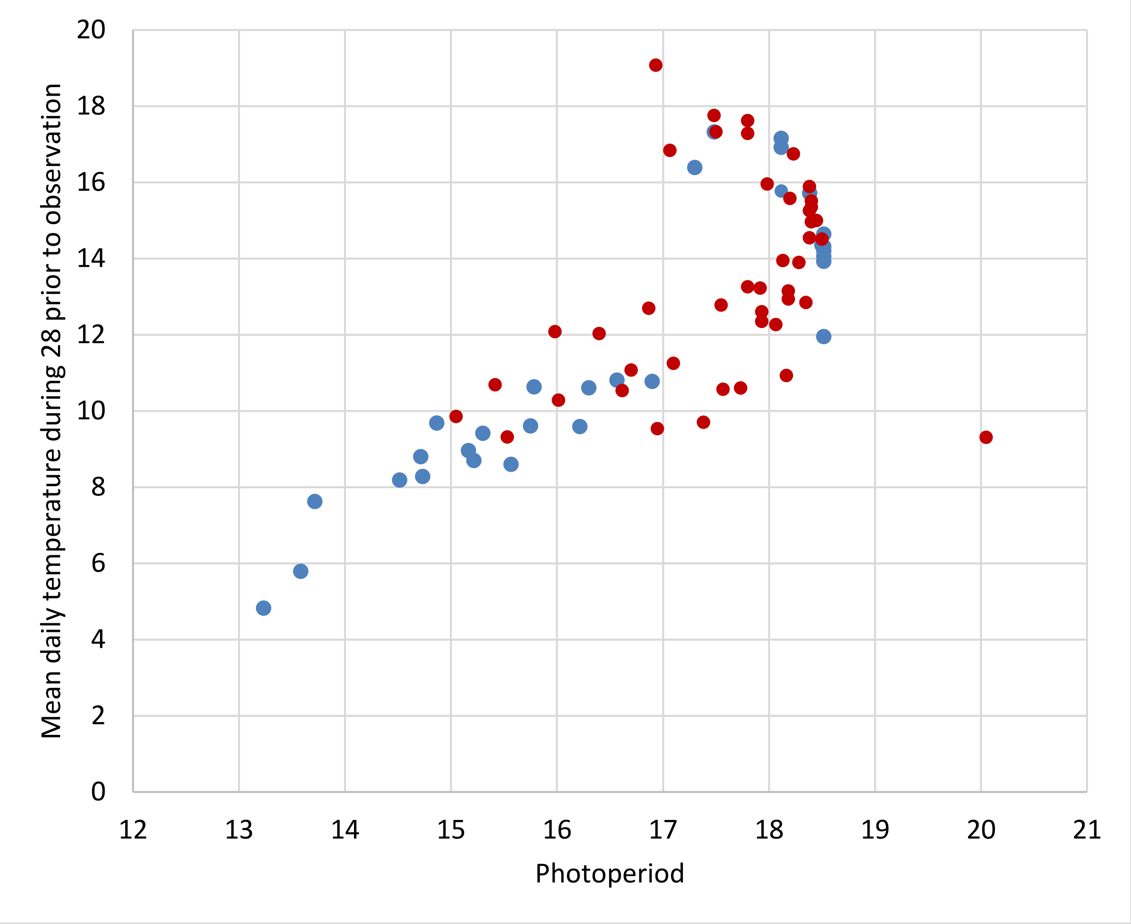


Figure 4.10: Adult *B. rufimanus* observations in traps between 2009 and 2014 at Crowland, Bourne and Tilney St. Lawrence with photoperiod on the day of observation and mean daily temperature during the 28 days prior to observation. Blue data point = zero *B. rufimanus* recorded per trap, red data point = 1 or more mean *B. rufimanus* recorded per trap.

4.3.2 *Oviposition*

At East Raynham in Norfolk in 2009 oviposition started in the earlier sown broad beans at very low levels before the date of the first assessment on 5 June and on this date, there were two pod-bearing nodes present in the beans. Pods were between two and five cm long and first pods were formed on 29 May. In the later sown beans there were fewer pods present, occurring only on the basal pod-bearing node on 5 June, and no eggs were present on the pods (Table 4.7). Mean maximum daily temperature in the week preceding first pod on 29 May for sowing one was 18.5°C, for the two days preceding first pod 19.2°C and for the two days inclusive of the day of first pod 21.1°C (Figure 4.11). Mean maximum daily temperature in the week preceding first pod on 5 June for sowing two was 17.7°C, for the two days preceding first pod 13.8°C and for the two days inclusive of the day of first pod 14.2°C. When the third and fourth assessments were carried out mean maximum daily temperature was higher than that preceding first pod formation, and oviposition on new pods was very low or did not occur (Figure 4.11). The total period of oviposition was 45 days.

Table 4.7: Mean number of *B. rufimanus* eggs recorded per pod on ten plants selected randomly within areas that received no insecticide applications, on four assessment dates in 2009 in broad beans at East Raynham, Norfolk. Standard error shows variation from the mean.

Date	Mean number of eggs per pod within sowing 1	Mean number of eggs per pod within sowing 2
05/06/09	0.256 ± 0.455	0
18/06/09	0.375 ± 0.202	0.303 ± 0.305
08/07/09	0.025 ± 0.069	0.020 ± 0.042
13/07/09	0	0.063 ± 0.092

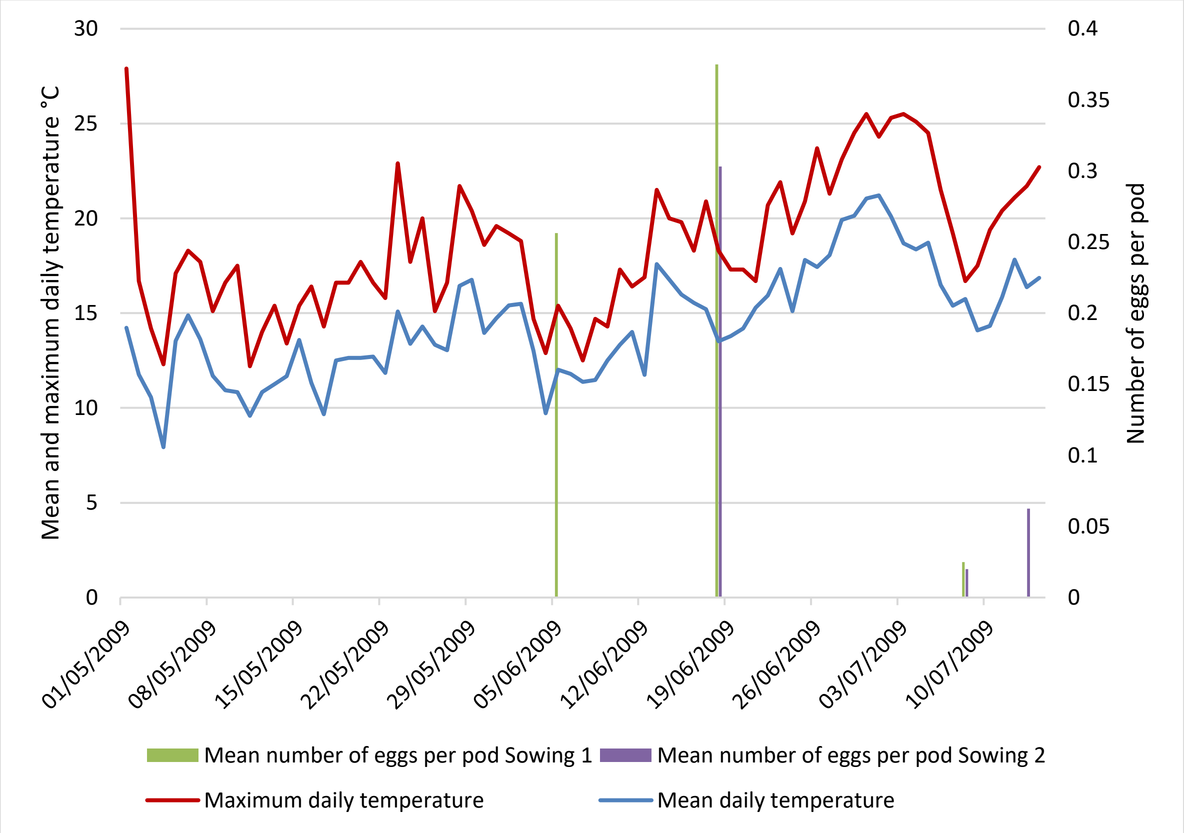


Figure 4.11: Number of *B. rufimanus* eggs recorded per pod in broad beans at East Raynham, Norfolk, with mean and maximum daily temperature. Oviposition was recorded on 5 June, 18 June, 8 July and 13 July on ten plants selected randomly within areas that received no insecticide applications.

4.4 Discussion

4.4.1 *Monitoring of adult B. rufimanus on emergence from overwintering sites*

There were only weak relationships between mean daily temperature and the number of *B. rufimanus* recorded in traps as they emerged from overwintering sites, although there were trends that emerged from the observations. Mean daily temperature in the 28 days prior to observations appeared to influence activity and emergence from overwintering sites. Emergence occurred between mid-April and mid-May during the years of the study, when photoperiod including civil twilight was between 15 hours and four minutes and 17 hours and 34 minutes. Although the optimal photoperiod for the termination of reproductive diapause in male adult *B. rufimanus* was found to be 18 hours under laboratory conditions (Tran and Huignard, 1992), emergence from overwintering sites in this study was not reliant on 18 hours photoperiod. *B. rufimanus* adults did not emerge from overwintering sites when photoperiod was less than 15 hours, indicating that photoperiod limited emergence only at this level. This may indicate that emergence occurred prior to the end of reproductive diapause and there are supporting reports that emergence occurs prior to the end of diapause (Roubinet, 2016) and that the females must feed on flowering *V. faba* to terminate diapause (Tran and Huignard, 1992). Huignard *et al.* (1990) provided some evidence that at the start of crop colonisation, male adults were able to reproduce, although females were still in reproductive diapause. This was confirmed by the examination of female reproductive organs and the stage at which vitellogenesis started. A short phase was recorded following emergence, during which vitellogenesis had not started, prior to 13 May in France in 1987 (Huignard *et al*., 1990).

Temperature may be a determining factor for emergence from overwintering sites, although this data does not confirm that it is. No adults emerged when mean daily temperature was below 9°C, possibly indicating a lower threshold below which emergence does not occur. Although there are reports that temperature thresholds are required for adults to colonise crops (Biddle and Cattlin, 2007; Leppik *et al.,* 2014; Roubinet, 2016), it is not yet possible to confirm this. Despite the lack of firm statistical confirmation here, the comparison of adult *B. rufimanus* recorded in traps with mean daily temperature showed trends that indicated that, in combination with a minimum photoperiod, higher temperature led to greater numbers of adults recorded (Tables 4.1 to 4.5). Both photoperiod and temperature are important for emergence, but whilst there is a specific threshold of 15 hours for photoperiod, once that has been achieved beetles will emerge, and with increasing temperature the number of adult *B. rufimanus* emerging appears to increase (Figure 4.9).

Semi-natural habitats are recognised as being important overwintering sites for the maintenance of beneficial species such as predators and parasitoids (Landis *et al*., 2000) but little is known about the role of these habitats for insect pest species and the of insect pest abundance in overwintering sites (Rusch *et al.,* 2012). Rusch *et al.* found that although temperature was likely to influence development rate in most insects, it did not tell the complete story about timing of adult emergence in the spring, which also involves the termination of seasonal diapause. Diapause termination occurs in response to external stimuli, and local conditions at the emergence site may affect emergence time, suggesting that environmental effects on emergence time are important (Rusch *et al*., 2012). Semi-natural habitats can support insect pest species during the winter and may act as a source of pest colonisation in crops (Leather *et al.,* 1993). Where an organism's survival is closely tied to the phenology of another species, there should be strong selection for the two to use the same cues, or at least cues that have historically been strongly correlated (Forrest and Thomson, 2011).

The attraction of the semio-chemical lures was sufficient to provide an indication of adult emergence prior to flowering in *V. faba*, but when flowering commenced the semiochemical lures were of little value for monitoring purposes. It was noted that the presence of *V. faba* flowers was not required to stimulate emergence of adults from overwintering sites, and in all years, emergence occurred prior to the onset of flowering. Further work is required to improve the effectiveness of semiochemical attractants for use in monitoring, and potentially for mass capture of adult *B. rufimanus* in *V. faba* crops. Recent developments in France (Leppik *et al.,* 2014) sought to investigate the use of combined leaf, flower and pod compounds with sticky traps for mass capture. Leppik *et al*. (2014) found that males were strongly attracted by the flower volatiles, while the females showed a moderate attraction. The attraction to the host plant effect was improved by the presence of males on the flowers and the results showed that the bruchids were attracted by the smell of *V. faba* at flowering stage when adults were sexually mature (Leppik *et al,* 2014). They found also that the semiochemical profile of *V. faba* changed over time, and that *B. rufimanus* was not attracted to the plants at the vegetative stage. Leppik *et al.* (2014) reported that the mixture of compounds used in their experiments competed very well with flowering crops and capture was possible during the crop flowering period, at high levels. A patent was filed to protect a mixture of compounds shown to attract *B. rufimanus* at high levels and is being developed for mass capture in France (Frerot and Leppik, 2015). The mixture contains the active compounds acetate, hex-3-en-1-yl, ocimene, linalool , beta-caryophyllene and limonene. The presence of the active compound hex-3-en-1-yl may reproduce the olfactory properties of *V. faba* pods and may attract *B. rufimanus* due to the necessity for adult females to locate pods on which to oviposit (Frerot and Leppik, 2015). This is currently being investigated further in the UK, to further develop a lure and kill management system for *B. rufimanus*, which includes in the mixture the entomopathogen *Beauveria bassiana.*

4.4.2 *Oviposition*

The study at East Raynham showed that oviposition occurred at very low levels within both the early and later sown *V. faba* areas. It appeared that maximum daily temperature immediately prior to oviposition was more critical for stimulation of oviposition than longer term mean maximum daily temperature, and eggs were not present at the assessment on 5 June on first pods of the second sowing following two days at low temperature. While temperature continued to be above 20°C for a considerable period following the formation of first pods and during further pod formation, much lower numbers of newly laid eggs were recorded after the second assessment on 18 June until 13 July when pods were full. This indicated that peak oviposition occurred during the initial two weeks and gradually declined for a further four weeks. The total period of oviposition at East Raynham was 45 days. Further study on a larger scale is required to fully investigate the factors influencing oviposition by *B. rufimanus.* This study is not large enough to draw any firm conclusions.

Chapter 5: The influence of temperature, crop development and insecticide applications on oviposition and damage caused by *B. rufimanus* in *V. faba*

5.1 Introduction

Pyrethroids are synthetic insecticides similar to natural pyrethrins, which disrupt the nervous system of insects leading to loss of co-ordination, paralysis and death. The mode of action disrupts the normal function of nerves via the voltage-sensitive sodium channels that initiate action potentials in nerve, muscle, and other excitable cells (Soderlund, 2010). Synthetic pyrethroids were developed in the 1960's and first registered in the 1970's, modified from natural pyrethrins, and evaluated for increased photo-stability, quick insecticidal activity and low to moderate mammalian toxicity (Soderlund, 2010). Several factors influence insecticide toxicity, one being temperature. Studies indicate that synthetic pyrethroids have a negative temperature coefficient, with higher toxicity to *Spodoptera littoralis* (Boisd.), a noctuid moth, at 20°C compared to 35°C for the active substances permethrin, fenvalerate, cypermethrin and deltamethrin (Riskallah, 1983). Harwood *et al*. (2009) found that a temperature reduction of 10°C, from 23°C to 13°C, led to increased toxicity of the pyrethroids permethrin and lambda-cyhalothrin for the species *Chironomus dilutus* (Shobanov, Kiknadze & Butler), a midge. Pyrethroid compounds have been widely used to control insect pests in UK crops since the 1970's, and many species have developed resistance to pyrethroids, including another pest of *V. faba*, the pea and bean weevil (Foster, 2015). There is inconclusive evidence that *B. rufimanus* may be developing resistance to pyrethroids in the UK (L. Smart, 2015, Personal Communication). Until 2017, pyrethroids have been the only active substances approved for use in UK *V. faba* crops for the control of beetle pests. Further restrictions are placed on the use of pyrethroids during the period of activity of *B. rufimanus*, while *V. faba* crops are flowering, and only two active substances, deltamethrin and lambda-cyhalothrin, are approved for use during this period. Lambda-cyhalothrin is the only active substance with on-label recommendation for use for the control of *B. rufimanus* in *V. faba* (FERA, 2016). Lambda-cyhalothrin has no ovicidal activity and thus insecticide applications target adult activity prior to oviposition.

B. rufimanus oviposits on pods of *V. faba* and eggs are laid singly and protected by a gelatinous substance exuded at oviposition to attach the egg firmly to the pod (Hoffmann *et al*., 1962; Southgate, 1979). Recent development of semiochemical attractants for mass capture of *B. rufimanus* in France indicates the strong attraction of the active compound hex-3-en-1-yl which reproduces the olfactory properties of *V. faba* pods and may attract *B. rufimanus* due to the necessity for adult females to locate pods on which to oviposit (Frerot and Leppik, 2015). A high degree of synchronisation of crop growth stage and attack by *B. rufimanus* in the host crop, may indicate that a great degree of precision is required when planning management of pest attack. Placement of eggs onto the pod is irregular, eggs are laid individually, and position is not related to pod characteristics or the position of the seeds inside the pod (Hoffmann et al., 1962; Ward, 1999). It is, however, related to the position of podded plant nodes and eggs are laid primarily on the lower nodes of plants that are present when oviposition begins. The difficulty of penetration into the lower part of the crop when applying insecticides leads to inadequate control of *B. rufimanus* in some instances. The period of peak oviposition occurs in the initial two weeks of oviposition but may last one to two months depending on region and climate (Hamani and Medjdoub-Bensaad, 2015). There is no point of exposure of the larvae on the pod surface (Hoffmann et al., 1962). Managing *B. rufimanus* pest attacks is difficult due to their mobility, the density of the crop at application, and the lack of persistence of pyrethroids at high temperatures (Mansoor *et al.,* 2015). Operational choices such as the use of reduced water volumes at application, to increase the speed at which operations can be undertaken, sprayer nozzle choice, and application of insecticides during the evening and early morning to avoid harm to beneficial organisms, may further reduce the level of efficacy of insecticide applications.

During the observations undertaken in this study, large non-randomised plots were used in preference to a small plot randomised block design, following experience undertaking small plot screening trials to test the efficacy of insecticides for control of *B. rufimanus*. It was found in previous studies that, due to the highly mobile nature of *B. rufimanus,* small plots were re-invaded very quickly following the application of contact-acting insecticides, reducing the effectiveness of insecticides in comparison to commercial field-scale practice (Ward, 1999). For species that are highly mobile, the results of field studies to evaluate the effects of insecticides may be misinterpreted, and there is a risk of concluding that there is no effect, when in fact the methodology was inadequate (Macfadyen *et al.,* 2014). Local movement of invertebrates is common in agroecosystems and is often a response to changes in resource availability and quality, which may be affected by insecticide use. Target pest movement must be considered when designing field studies or observations but this presents a great challenge, when considering highly mobile species, to design experiments in which it is possible to make inferences using statistical analysis. Field studies using replicated plots in a randomized complete block design are frequently used and allow inferential statistical analyses, although plot sizes are often too small for effective study of the target organism. Movement may occur between plots, meaning that plots are not statistically independent from the other, which may lead to misleading conclusions. An experimental design that would provide a more robust indication of insecticide efficacy for mobile species would be one in which each treatment plot is as large as possible with all treatments fully replicated. However, this is often practically impossible, particularly when studies are conducted with the assistance of farmers. Some studies suggest that larger plots with reduced replication are better than small plots for assessing the effects of insecticides on mobile species (Smart *et al*. 1989) and that rapid reinvasion of small plots following treatment means that only short-term effects can be monitored (Macfadyen *et al.,* 2014). Split-field studies may be an appropriate method of conducting trials for mobile species in commercial crops, although this may limit the level of control that the researcher has over agronomic inputs and timing. It may be difficult to infer conclusions from the results of such trials when reduced replication is used. It is possible that field trials of the design described in this study may be considered to be useful only for the purpose of demonstration, the emphasis being on observed impact, not on measured results that are critically compared. Although this type design is often aimed at observed comparison, the treatment being a unique experimental practice or effect, some measurements may be gathered for comparison. Measurements may be taken at various locations within a strip to account for in-field variation and careful interpretation is required to avoid drawing the wrong conclusions from random differences between treatments (Alberta Agriculture and Forestry, 2017).

The aims of this study were to observe the effects of temperature, crop development and insecticide applications on oviposition and damage caused by *B. rufimanus*. The work was undertaken as part of two projects. The first was funded by the Department of Environment, Food and Rural Affairs and the Horticultural Development Council (now AHDB-Horticulture), with partners Frontier Agriculture, Velcourt Group Ltd., Syngenta Crop Protection UK, the National institute of Agricultural Botany, Wherry and Sons Ltd., the Processors and Growers Research Organisation, Nickerson-Advanta Ltd., Raynham Farming Company, Rothamsted Research, CPB-Twyford Ltd. and Bayer Crop Science Ltd, under the Sustainable Arable Link program (project number LK09102, ‘Integrated Control of the Bean

Seed Beetle, *Bruchus rufimanus*'). The second was co-funded by the Processors and Growers Research Organisation, Syngenta Crop Protection UK, Frontier Agriculture, Oecos, Innovate UK, DEFRA and BBSRC with academic partner Rothamsted Research and subcontractor Velcourt Group Ltd. who provided access to field trial sites (Project number 100871, 'A Novel Monitoring and Forecasting System for the Integrated Management of Bean Seed Beetle, *Bruchus rufimanus*').

Investigations were undertaken to observe the factors influencing oviposition and damage caused by *B. rufimanus* during the formation and development of *V. faba* pods. A model available in France, where similarly damaging incidence of *B. rufimanus* is experienced (F. Muel, 2006, Personal Communication), uses temperature to predict optimum spray date. The system forecasts periods of high *B. rufimanus* oviposition, when temperature is above 20°C, and recommends sprays to coincide with this. Uncertainty about the appropriateness of such a system in the UK, given the evidence that *B. rufimanus* can adapt its developmental strategies to survive in a variety of climatic and geographical areas (Medjdoub-Bensaad *et al.,* 2007), led to an investigation to establish whether a similar commercial system and threshold could be employed in the UK.

5.2 Methods

5.2.1 *Field trials to establish the effect of crop development, insecticide applications and temperature on activity and damage*

Field trials were established between 2009 and 2013 to evaluate the effects of crop development, number of insecticide applications and temperature on damage caused to *V. faba* by *B. rufimanus.* Trials were carried out in commercial spring sown *V. faba* crops at Aylmer Hall, Tilney St. Lawrence, Kings Lynn (Grid reference TF552148), using large field-scale plots 2.16 hectares in size for each treatment, using the commercial field bean cultivar Fuego, and tractor mounted sprayers with grower assistance. Using large plots, it was intended that there would be independence between the subsamples collected from each plot, to allow data analysis to be carried out, while recognising the limitations of unreplicated experimental designs for field trials. Plot size and design of trials were selected to allow the grower to carry out applications using field equipment, sprayers in this instance having a 36 metre boom width. In 2009, a trial was also carried out at Swanton Farms, Lydden, Dover (Grid reference TR243448), again using large unreplicated plots. The active ingredient lambda-cyhalothrin, a contact-acting pyrethroid insecticide, was applied to treated plots, to target adult *B. rufimanus,* at the full field rate of 0.075 litres per hectare in all years, diluted in 200 litres per hectare water and using standard flat fan spray nozzles to represent commercial practice (Tables 5.1 and 5.2). In 2011 and 2012 the active ingredient deltamethrin was used in an additional application within one treatment at the full field rate of 0.3 litres per hectare, diluted in 200 litres per hectare water and using standard flat fan spray nozzles (Tables 5.3 and 5.4). Plots were not replicated, and treatments were located in the same field for each experiment, location changing each year within a standard 5 year arable rotation. Temperature was monitored during crop growth using Tinytag° data loggers, and crop growth stage at application was recorded (Knott, 1990) (Appendix A).

The trials at Tilney St. Lawrence and Lydden in 2009 contained three treatments, one untreated control plot and two sprayed treatments (Table 5.1). Two insecticide applications (A1 and A2) were made to treatments 2 and 3 and treatment 1, as a control treatment, had no insecticide applications. The first insecticide applications for treatment 2 at Tilney St. Lawrence and Lydden were made when five flowering racemes were present on *V. faba* plants and for treatment 3 when the first pods had formed.

Table 5.1: Active ingredient, crop growth stage at application and dates of insecticide applications for trials at Tilney St. Lawrence and Lydden in 2009.

Treatment	Active ingredient	Location	Date of A1	Date of A2
1	Untreated		-	-
2	Lambda-cyhalothrin	Tilney St. Lawrence	3 June (5 flowering racemes)	16 June (A1 + 13 days (first pod formation))
		Lydden	1 June (5 flowering racemes)	12 June (A1 + 11 days (first pod formation))
3	Lambda-cyhalothrin	Tilney St. Lawrence	16 June (First pod formation)	2 July (A1 + 16 days)
		Lydden	15 June (First pod formation)	24 June (A1 + 9 days)

The trial at Tilney St. Lawrence in 2010 consisted of four treatments, one untreated control plot and three sprayed treatments (Table 5.2). Treatment 1 was unsprayed, treatment 2 had a single insecticide application at first pod formation and treatment 3 had two insecticide applications, the first made at first pod formation and the second 13 days later. Treatment 4 had a single insecticide application made 26 days after the formation of first pods.

Table 5.2: Active ingredient, crop growth stage at application and dates of insecticide applications for the trial at Tilney St. Lawrence in 2010.

Treatment	Active ingredient	Date of A1	Date of A2
1	Untreated	-	-
2	Lambda-cyhalothrin	23 June (First pod formation)	-
3	Lambda-cyhalothrin	23 June (First pod formation)	6 July (A1 + 13 days)
4	Lambda-cyhalothrin	19 July (First pod formation+ 26 days)	-

The trial at Tilney St. Lawrence in 2011 consisted of four treatments, one untreated control plot and three sprayed treatments (Table 5.3). Treatment 1 was unsprayed, treatment 2 had two insecticide applications, the first made at first pod formation and the second 13 days later. Treatment 3 repeated this with the requirement for 20^{o}C for two consecutive days prior to application of the first spray. This was not required for treatment 2. Treatment 4 had three insecticide applications, the first at first pod formation using the active ingredient lambda-cyhalothrin after a period of two consecutive days at 20^{o}C, the second 13 days later, again with lambda-cyhalothrin, and the third eight days after A2 using the active ingredient deltamethrin at 0.3 litres per hectare.

Table 5.3: Active ingredient, crop growth stage at application and dates of insecticide applications for the trial at Tilney St. Lawrence in 2011.

Treatment	Active ingredient	Date of A1	Date of A2	Date of A3
1	Untreated	-	-	-
2	Lambda-cyhalothrin (20°C not required at A1)	03 June (First pod formation)	16 June (A1 + 13 days)	-
3	Lambda-cyhalothrin (20°C required at A1)	03 June (First pod formation)	16 June (A1 + 13 days)	-
4	Lambda-cyhalothrin at A1 and A2 and deltamethrin at A3 (20°C required at A1)	03 June (First pod formation)	16 June (A1 + 13 days	24 June (A2 + 8 days)

The trial at Tilney St. Lawrence in 2012 consisted of four treatments, one untreated control plot and three sprayed treatments (Table 5.4). Treatment 1 was unsprayed. Treatment 2 had two insecticide applications, the first made as close to first pod formation as possible and without the requirement for a period of two consecutive days at 20^{o}C, with the second application 14 days later. Treatment 3 had two insecticide applications, the first made when temperature had reached 20°C for two consecutive days when pods were formed and the second application 14 days later. Treatment four had three insecticide applications, the first made when temperature had reached 20°C for two consecutive days when pods were formed using the active ingredient lambda-cyhalothrin, the second made 14 days later, again with lambda-cyhalothrin, and the third 15 days after A2 using the active ingredient deltamethrin at 0.3 litres per hectare.

Table 5.4: Active ingredient, crop growth stage at application and dates of insecticide applications for the trial at Tilney St. Lawrence in 2012.

Treatment	Active ingredient	Date of A1	Date of A2	Date of A3
1	Untreated	-	-	-
2	Lambda-cyhalothrin (20°C not required at A1)	19 June (9 days after first pod formation)	03 July (A1 + 14 days)	-
3	Lambda-cyhalothrin (20°C required at A1)	25 June (15 days after first pod formation)	09 July (A1 + 14 days	-
4	Lambda-cyhalothrin at A1 and A2 and deltamethrin at A3 (20°C required at A1)	25 June (15 days after first pod formation)	09 July (A1 + 14 days	24 July (A2 + 15 days)

The trial at Tilney St. Lawrence in 2013 consisted of three treatments, one untreated control plot and two sprayed treatments (Table 5.5). Treatment 1 was unsprayed. Treatment 2 had two insecticide applications, the first made at first pod formation without the requirement for a period of two consecutive days at 20°C, and the second application 14 days later. Treatment 3 had two insecticide applications, the first made when temperature had reached 20°C for two consecutive days when pods were formed and the second application 14 days later.

Table 5.5: Active ingredient, crop growth stage at application and dates of insecticide applications for the trial at Tilney St. Lawrence in 2013.

Treatment	Active ingredient	Date of A1	Date of A2
1	Untreated	-	-
2	Lambda-cyhalothrin (20°C not required at A1)	19 June (first pod formation)	03 July (A1 + 14 days)
3	Lambda-cyhalothrin (20°C required at A1)	25 June (First pod formation)	09 July (A1 + 14 days

All trials from 2009 to 2012 were harvested at the mature dry stage using commercial combine harvesters and four bulk grain samples were taken directly from the harvester at different locations from each plot, including unsprayed plots, to provide replicated subsamples for analysis of damage caused by *B. rufimanus*. At Tilney St. Lawrence in 2009, five bulk samples were taken from each plot at harvest. Both damaged and undamaged samples for bulk samples in all years were weighed using a Mettler BC4000 balance to determine the proportion of seed damage. Damage included round holes 3 to 4 mm in diameter caused as adult *B. rufimanus* emerged following pupation, brown marks on the seed surface caused as the larvae moved under the testa during early seed invasion, entry wounds on the surface of the seed caused as the larvae burrowed into the seed and translucent circles on the surface of the seed under which adults remained (Plate 5.1). If necessary seed was cut open to confirm the presence of *B. rufimanus* adults or larvae.

At Tilney St. Lawrence in 2010 an additional assessment was carried out on 75 plants from each large plot when plants were at desiccation stage immediately prior to harvesting, to determine the level of damage per plant. A similar assessment was repeated in 2013 on 15 plants per plot. Pods were removed from the plants at each node, grains removed, counted and recorded. Grains were examined for evidence of damage caused by *B. rufimanus* as described for bulk samples (Plate 5.1) and damaged grains counted and recorded. Data were summarised for both bulk samples and plant samples as proportion of total sample size, either by weight for bulk samples, or by number of grains for plant samples.

Prior to analysis, data were examined for normality and homogeneity, using the Anderson-Darling and Levene's Tests, respectively. Data for mean proportion of damaged grains per treatment were transformed using arcsine transformation. Analysis of variance was carried out for data from trials in 2010 and 2013, where a greater number of subsamples were taken from each plot, to determine whether there were differences between treatment programs. It was recognised that conclusions from the analyses of bulk samples from trials should be treated with extreme caution due to trial layout and reduced replication within plots, and only descriptive statistics are given here.

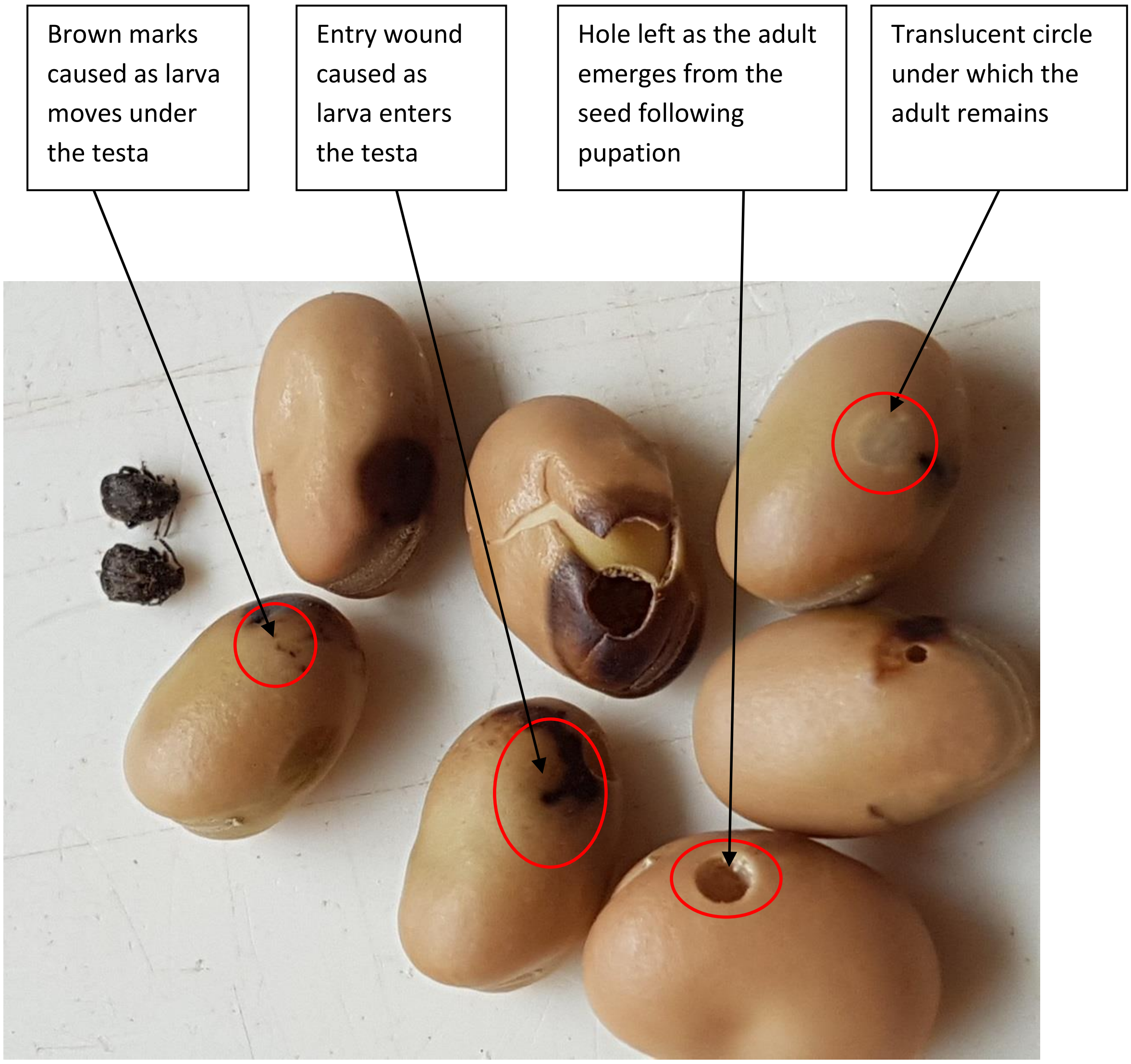


Plate 5.1: Damage to *V. faba* caused as adult *B. rufimanus* emerge following harvest. Holes are 3 to 4 mm wide and brown marks are caused as larvae burrow into the seed and move underneath the seed coat. Translucent circles are seen on the testa when adult *B. rufimanus* has not emerged.

5.2.2 *The influence of temperature on oviposition*

Studies of *B. rufimanus* oviposition were undertaken at Tilney St. Lawrence in 2012, 2013 and 2014 to observe the factors influencing the level of oviposition during the period of *V. faba* pod formation and development. The number of eggs per pod was recorded on 20 plants in an area of a commercial *V. faba* crop that received no insecticide applications for *B. rufimanus* control. Assessments were carried out on one occasion at the growth stage when all pods were formed and oviposition continued to occur, but before eggs dropped from the pods. All eggs were recorded, including those that had clearly been present on the pod from the commencement of oviposition and those that were newly laid (Chapter 4, Plates 4.2 and 4.3), to determine total levels of oviposition, and oviposition was recorded for each pod-bearing plant node. Data were examined for normality and homogeneity, using the Anderson-Darling and Levene’s Tests, respectively. Data were analysed using SAS University Edition® to examine difference between years using the Kruskal-Wallis non-parametric test. Pairwise two-sided comparison analysis was carried out using the Dwass, Steel, Critchlow-Fligner Method.

5.3 Results

5.3.1 *Field trials to establish the effect of crop development, insecticide applications and temperature on activity and damage*

One way analysis of variance was carried out on data recorded for *B. rufimanus* damage to grains during the plant assessment data at Tilney St. Lawrence in 2010 (Table 5.7). Box plot and descriptive statistics were derived for proportion of damaged grains per treatment (Figure 5.1; Table 5.6) and data were analysed using arcsine transformed mean proportion of damaged grains per treatment (Table 5.7).

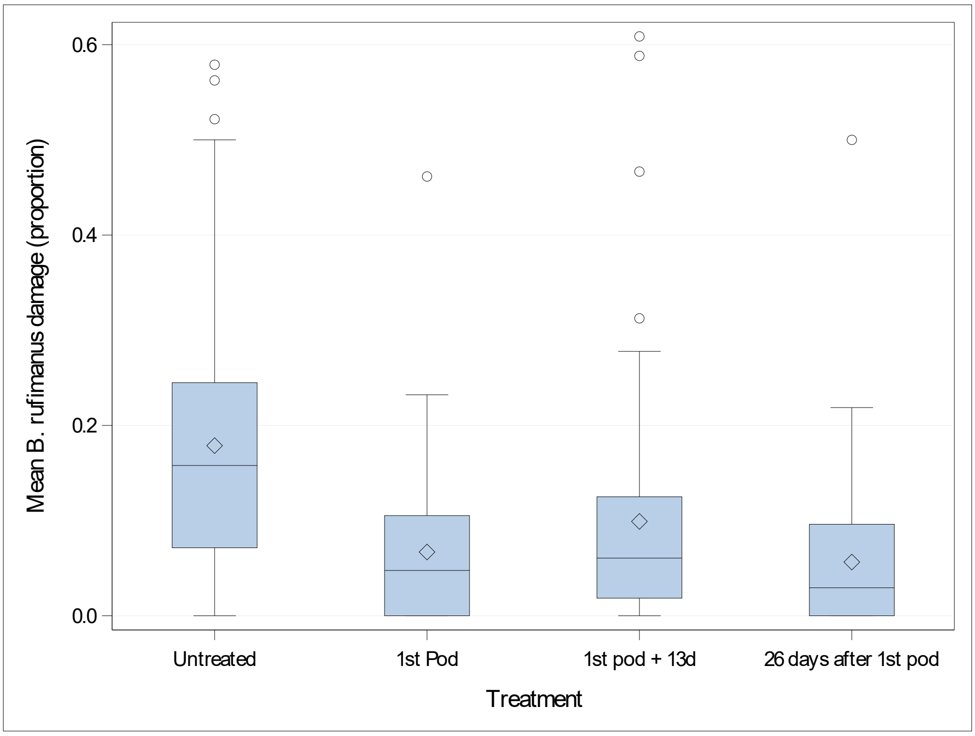


Figure 5.1: Mean *B. rufimanus* damaged grains per treatment, recorded as proportionate number of grains, at Tilney St. Lawrence in 2010. Number of observations per treatment is 75 plants. Whiskers represent minimum values and 1.5 X Interquartile Range where suspected outliers are represented as unfilled circles.

Table 5.6: Descriptive statistics for mean *B. rufimanus* damaged grains per treatment, recorded as proportionate number of grains, at Tilney St. Lawrence in 2010, with mean total grains per plant and mean damaged grains per plant for each treatment. 75 plants were assessed per treatment. Coefficient of Variation is given for each treatment.

Descriptive statistics					95% confidence interval for mean				
Treatment	N	Mean proportion damaged grain	Standard deviation	Standard Error	Lower bound	Upper bound	Min	Max	CV%
Untreated	75	0.1788	0.1302	0.0150	0.1488	0.2087	0	0.5789	72.84
1st pod only	75	0.0670	0.0771	0.0089	0.0492	0.0847	0	0.4615	115.09
1st pod + 13 days	75	0.0991	0.1232	0.0142	0.0708	0.1275	0	0.6087	124.35
26 days after 1st pod	75	0.0564	0.0797	0.0092	0.0381	0.0748	0	0.5000	141.16

Treatment	Mean number of grains per plant	Mean number of damaged grains per plant
Untreated	28.48	5.13
1st pod only	28.89	2.05
1st pod + 13 days	27.85	2.73
26 days after 1st pod	29.29	1.72

Table 5.7: Analysis of variance of mean *B. rufimanus* damaged grains (arcsine transformed) per treatment, recorded as proportionate number of grains, at Tilney St. Lawrence in 2010. 75 plants were assessed per treatment.

	DF	Sum of squares	Mean square	F value	P
Treatment	3	2.3780	0.7927	23.15	<.0001
Error	296	10.1354	0.0342		
Corrected total	299	12.5133			
Treatment	Mean	N	Group*		
Untreated	0.4059	75	a		
1st pod only	0.2075	75	bc		
1st pod + 13 days	0.2603	75	b		
26 days after 1st pod	0.1725	75	c		

*Means in this column, with the same letter, are not significantly different ($p>0.05$).

Analysis of Variance showed that there were statistically significant differences between treatments (Table 5.7), although this conclusion should be treated with caution, as there was a risk that data for each plot were not independent. There appears to be a significant difference in *B. rufimanus* damage between the untreated plot and all treatments at Tilney St. Lawrence in 2010, but the differences between the treated plots were more difficult to explain. The application at 26 days after first pod formation appeared to provide greater control of *B. rufimanus* than the two spray program.

Descriptive statistics and box plot for damage recorded on bulk subsamples taken from the trial at Tilney St. Lawrence in 2010 are shown in Table 5.8 and Figure 5.2. Greater variability from mean proportion of damage between subsamples within treatments was found between plants than between bulk samples (Tables 5.6 and 5.8). There were differences in the number of pods formed per plant due to the normal indeterminate growth of *V. faba,* which may have led to greater variability from mean values per treatment.

Damage was recorded by number of damaged grains. Bulk samples were weighed and size of sample not related to the number of pods formed.

Table 5.8: Descriptive statistics for mean *B. rufimanus* damaged grains per treatment, recorded as proportionate weight, at Tilney St. Lawrence in 2010, with mean total weight of grains per subsample and mean weight of damaged grains per subsample for each treatment. 4 bulk subsamples were assessed per treatment. Values are given for Coefficient of Variation.

Descriptive statistics					95% confidence interval for mean				
Treatment	N	Mean proportion damaged grain	Standard deviation	Standard Error	Lower bound	Upper bound	Min	Max	CV%
Untreated	4	0.0595	0.0125	0.0063	0.0396	0.0794	0.0461	0.0702	21.02
1st pod only	4	0.0463	0.0056	0.0028	0.0373	0.0552	0.0401	0.0537	12.13
1st pod + 13 days	4	0.0604	0.0151	0.0075	0.0364	0.0844	0.0488	0.0821	24.97
26 days after 1st pod	4	0.0883	0.0289	0.0145	0.0422	0.1343	0.0617	0.1284	32.80

Treatment	Mean weight of grain per subsample (g)	Mean weight of damaged grain per subsample (g)
Untreated	1487.28	87.72
1st pod only	1755.05	81.19
1st pod + 13 days	1685.35	10217
26 days after 1st pod	1659.62	150.71

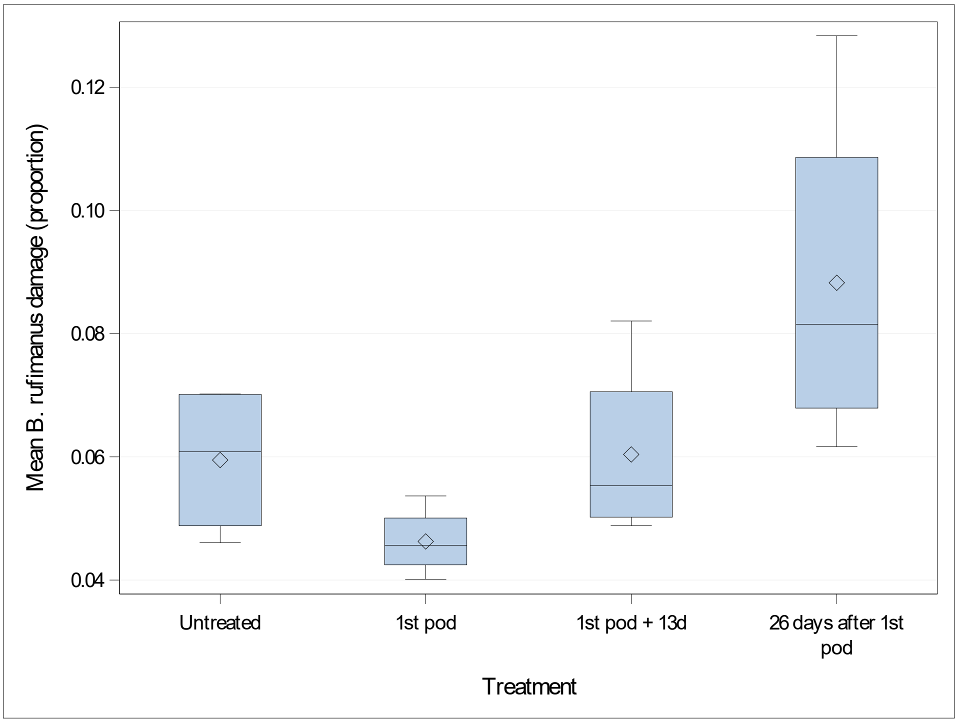


Figure 5.2: Mean *B. rufimanus* damage per treatment, recorded as proportionate weight, at Tilney St. Lawrence in 2010. Number of observations per treatment is 4 bulk subsamples. Whiskers represent maximum and minimum values.

Temperature was greater than 20°C for two days preceding all spray applications at Tilney St. Lawrence in 2010 (Figure 5.3).

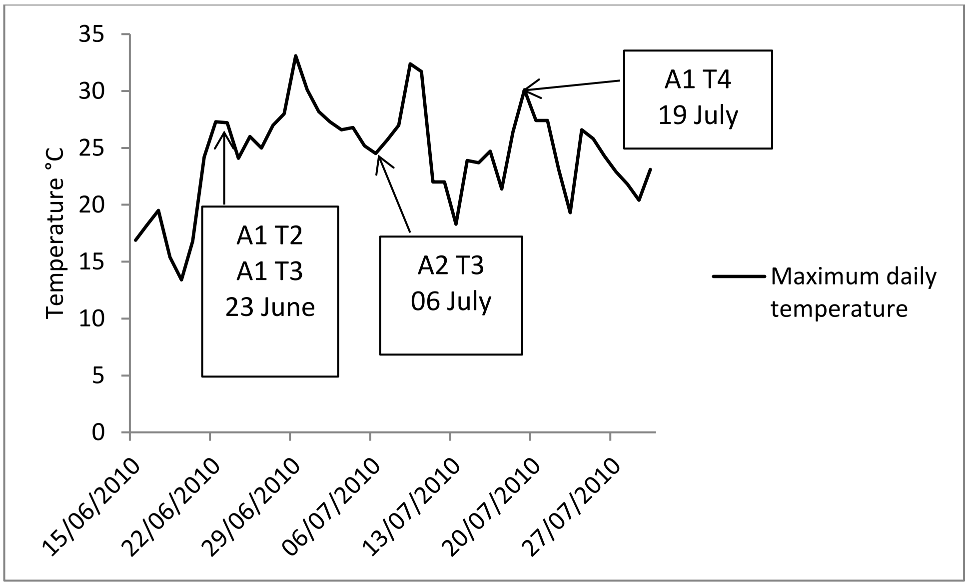


Figure 5.3: Maximum daily temperature at Tilney St. Lawrence in June and July 2010. Arrows indicate spray applications – application 1 (A1) for treatment 2, 23 June at first pod, application 1 (A1) for treatment 3, 23 June at first pod and application 2 (A2), 6 July, application 1 (A1) for treatment 4, 19 July.

The bulk subsamples from Tilney St. Lawrence in 2010 showed a different pattern of damage between plots, with the untreated plot having approximately the same level of damage as the plot in which two sprays were applied. Where a single spray was applied at first pod only, the proportion of damage appeared to be lower, and where a single application was made 26 days after first pod, the proportion of damage was higher (Figure 5.2; Table 5.8).

One way analysis of variance was carried out on the plant assessment data from Tilney St. Lawrence in 2013 (Table 5.10). Box plot and descriptive statistics are provided for proportion of *B. rufimanus* damaged grains per treatment (Figure 5.4; Table 5.9) and data were analysed using arcsine transformed mean proportion of damaged grains per treatment (Table 5.10). No bulk subsamples were taken in 2013.

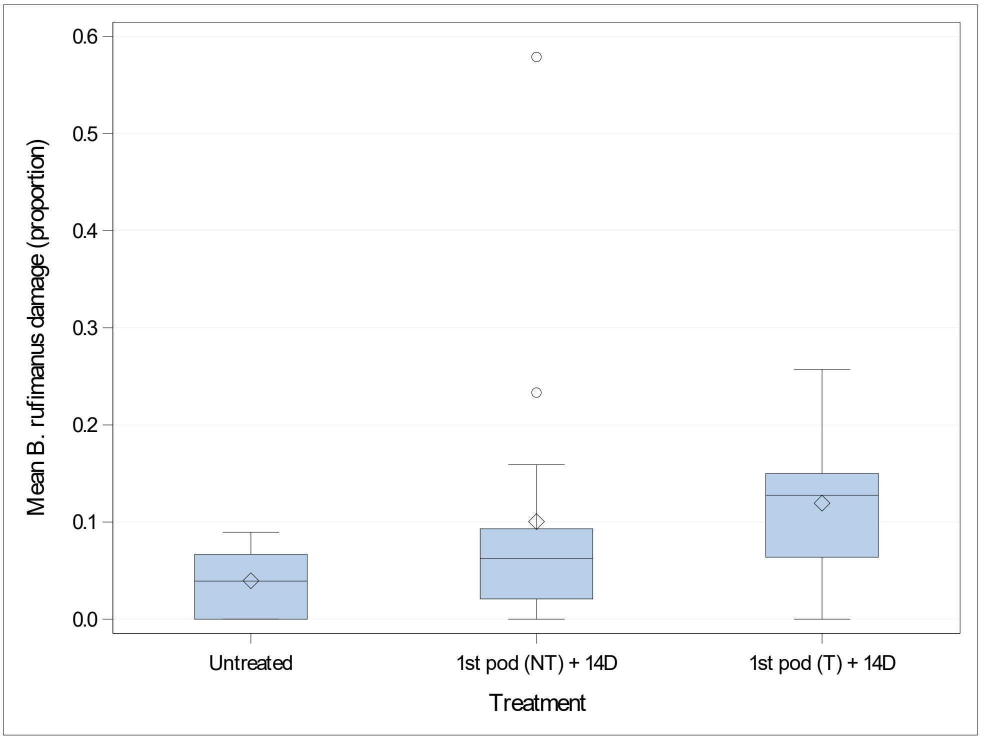


Figure 5.4: Mean *B. rufimanus* damage per treatment, recorded as proportionate number of grains, at Tilney St. Lawrence in 2013. Number of observations per treatment is 15 plants. Whiskers represent maximum and minimum values or 1.5 X Interquartile Range where suspected outliers are represented as unfilled circles. T = two consecutive days at 20°C required for spray application, NT = no temperature requirement.

Table 5.9: Descriptive statistics for mean *B. rufimanus* damaged grains per treatment, recorded as proportionate number of grains per plant, at Tilney St. Lawrence in 2013, with mean total grains per plant and mean damaged grains per plant for each treatment. 15 plants were assessed per treatment. Values are given for Coefficient of Variation.

Descriptive statistics					95% confidence interval for mean				
Treatment	N	Mean proportion damaged grain	Standard deviation	Standard Error	Lower bound	Upper bound	Min	Max	CV%
Untreated	15	0.0396	0.0325	0.0084	0.0215	0.0576	0	0.0896	82.21
1st pod + 14 days (without temperature requirement)	15	0.1005	0.1459	0.0377	0.0197	0.1813	0	0.5789	145.19
1st pod + 14 days (with temperature requirement)	15	0.1195	0.0744	0.0192	0.0782	0.1606	0	0.2571	62.33

Treatment	Mean number of grains per plant	Mean number of damaged grains per plant
Untreated	52.20	2.27
1st pod + 14 days (without temperature requirement)	43.20	3.27
1st pod + 14 days (with temperature requirement)	47.13	5.40

In 2013 there was a higher number of grains per plant than in 2010, which may have been due to weather conditions. In 2013 there was a higher number of grains per plant in the untreated plot compared to the treated plots (Table.5.9), although this did not occur in 2010. While this was observational, there was also a lower level of damage in the untreated plots compared to the treated plots (Figure 5.4, Table 5.9). Although definite conclusions may not be drawn, it should be considered whether spray applications in 2013 caused damage to beneficial organisms, pollinators and natural predators, leading to reduced pod set on treated plots and greater damage by *B. rufimanus*. Further study would be advisable to determine the effect of treatments on beneficial organisms.

Table 5.10: Analysis of variance of mean *B. rufimanus* damaged grains (arcsine transformed) per treatment, recorded as proportionate number of grains, at Tilney St. Lawrence in 2013. 15 plants were assessed per treatment.

	DF	Sum of squares	Mean square	F value	P
Treatment	2	0.2199	0.1099	4.35	0.0191
Error	42	1.0604	0.0252		
Corrected total	44	1.2803			
Treatment	Mean	N	Group*		
Untreated	0.1622	15	b		
1st pod + 14 days (without temperature requirement)	0.2749	15	ab		
1st pod + 14 days (with temperature requirement)	0.3302	15	a		

*Means in this column, with the same letter, are not significantly different ($p>0.05$).

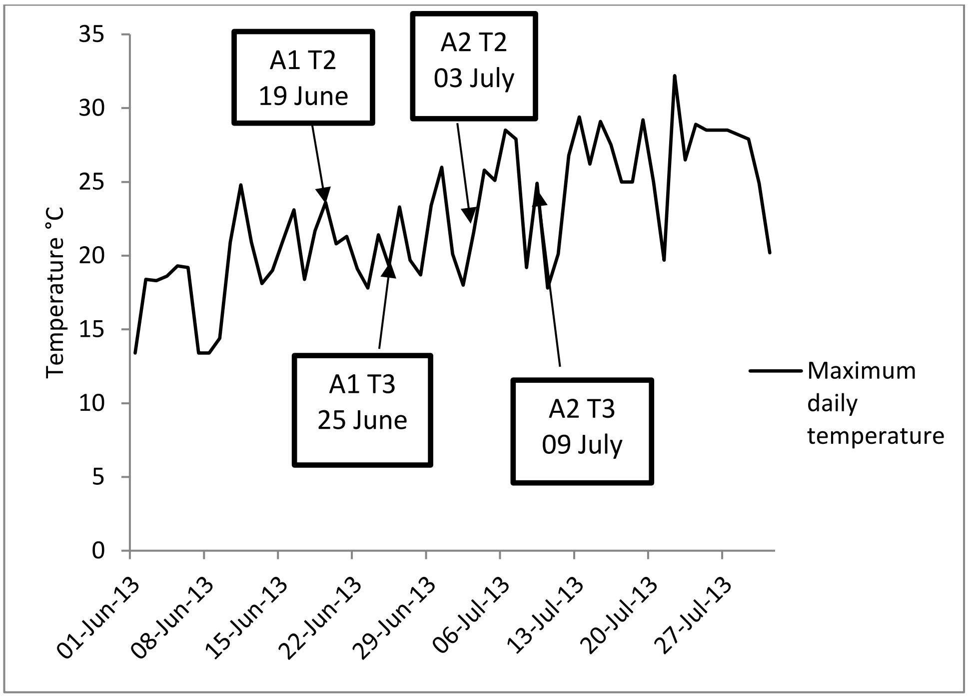


Figure 5.5: Maximum daily temperature at Tilney St. Lawrence in June and July 2013. Arrows indicate spray applications – application 1 (A1) for treatment 2, 19 June at first pod and application 2 (A2), 03 July, application 1 (A1) for treatment 3, 25 June at first pod and application 2 (A2), 09 July.

Temperature at the first application for Treatment 2 had reached 20°C prior to application, but not for two consecutive days (Figure 5.5). Temperature prior to the first application to Treatment 3 had reached 20°C for two consecutive days.

At Tliney St. Lawrence in 2013 the untreated plot had the lowest proportion of damage to grains by *B. rufimanus* compared to the treated plots (Figure 5.4; Table 5.8) and analysis of variance showed that the plant samples from the untreated plot had a significantly lower proportion of damage than treatment 3, the plot sprayed twice when a temperature threshold was reached (Table 5.10). There was no significant difference in damage between the two sprayed plots.

Descriptive statistics and box plot for damage recorded on bulk subsamples taken from the trial at Tilney St. Lawrence in 2009 are shown in Table 5.11 and Figure 5.6. The same are shown for damage recorded at Lydden in 2009 in Table 5.12 and Figure 5.8. Maximum daily temperature in 2009 is shown for Tilney St. Lawrence in Figure 5.7 and at Lydden in Figure 5.9.

Table 5.11: Descriptive statistics for mean *B. rufimanus* damaged grains per treatment, recorded as proportionate weight, at Tilney St. Lawrence in 2009, with mean total weight of grains per subsample and mean weight of damaged grains per subsample for each treatment. 5 bulk subsamples were assessed per treatment. Coefficient of Variation is given for each treatment.

Descriptive statistics					95% confidence interval for mean				
Treatment	N	Mean proportion damaged grain	Standard deviation	Standard Error	Lower bound	Upper bound	Min	Max	CV%
Untreated	5	0.0433	0.0147	0.0066	0.0251	0.0616	0.0327	0.0680	33.88
5 flowering racemes + 13 days	5	0.0160	0.0080	0.0036	0.0061	0.0259	0.0045	0.0258	49.90
1st pod + 16 days	5	0.0275	0.0077	0.0034	0.0180	0.0371	0.0192	0.0377	27.99

Treatment	Mean weight of grain per subsample (g)	Mean weight of damaged grain per subsample (g)
Untreated	928.08	39.08
5 flowering racemes + 13 days	865.92	14.09
1st pod + 16 days	1117.54	31.29

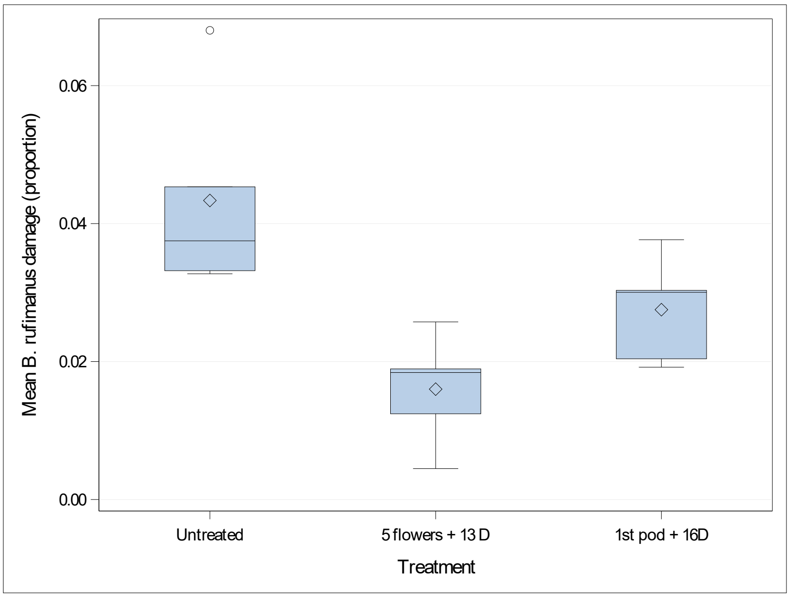


Figure 5.6: Mean *B. rufimanus* damage per treatment, recorded as proportionate weight, at Tilney St. Lawrence in 2009. Number of observations per treatment is 5 bulk subsamples. Whiskers represent maximum and minimum values.

At Tliney St. Lawrence in 2009 there was a higher proportion of damage on the untreated plot compared to the treated plots and the two spray program starting at 5 flowering racemes (treatment 2) (Table 5.11; Figure 5.6). The two spray program starting at 1st pod formation (treatment 3) had a higher proportion of damage than treatment 2.

Temperature reached or exceeded 20°C for two consecutive days at Tilney St. Lawrence prior to the first insecticide application for treatments 2 and 3 in 2009 (Figure 5.7).

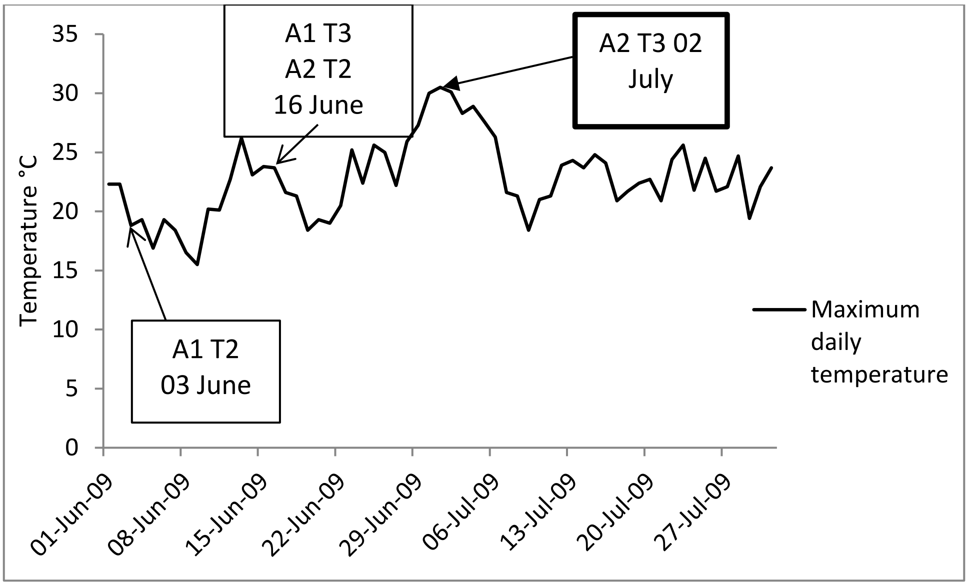


Figure 5.7: Maximum daily temperature at Tilney St. Lawrence in June and July 2009. Arrows indicate spray applications – application 1 (A1) for treatment 2, 03 June at 5 flowering racemes and application 2 (A2), 16 June, application 1 (A1) for treatment 3, 16 June at first pod and application 2 (A2) on 02 July.

Table 5.12: Descriptive statistics for mean *B. rufimanus* damaged grains per treatment, recorded as proportionate weight, at Lydden in 2009, with mean total weight of grains per subsample and mean weight of damaged grains per subsample for each treatment. 4 bulk subsamples were assessed per treatment. Coefficient of Variation is given for each treatment.

Descriptive statistics					95% confidence interval for mean				
Treatment	N	Mean proportion damaged grain	Standard deviation	Standard Error	Lower bound	Upper bound	Min	Max	CV%
Untreated	4	0.0047	0.0011	0.0006	0.0029	0.0065	0.0031	0.0058	24.04
5 flowering racemes + 11 days	4	0.0066	0.0040	0.0020	0.0002	0.0129	0.0037	0.0124	60.93
1st pod + 9 days	4	0.0112	0.0017	0.0009	0.0085	0.0140	0.0087	0.0126	15.39

Treatment	Mean weight of grain per subsample (g)	Mean weight of damaged grain per subsample (g)
Untreated	1161.04	5.43
5 flowering racemes + 11 days	1327.02	8.68
1st pod + 9 days	1207.13	13.54

At Lydden in 2009 there was a higher proportion of damage on the plot sprayed twice starting at 1st pod (treatment 3) compared to the untreated plot and the plot sprayed twice starting a 5 flowering racemes (treatment 2) (Table 5.12; Figure 5.8).

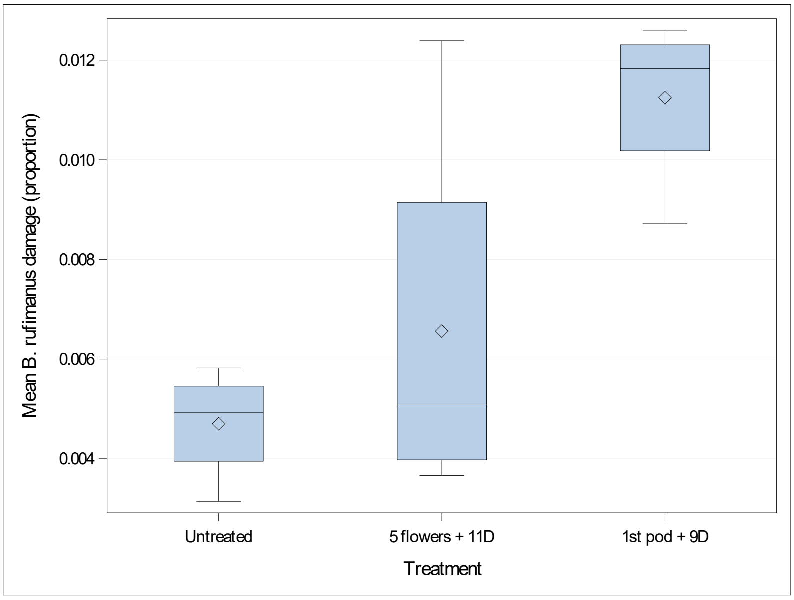


Figure 5.8: Mean *B. rufimanus* damage per treatment, recorded as proportionate weight, at Lydden in 2009. Number of observations per treatment is 4 bulk subsamples. Whiskers represent maximum and minimum values.

When A1 was applied at Lydden at five flowering racemes (treatment 2) the temperature had not reached 20°C for two days although this had been reached when A1 was applied to treatment 3 at first pod formation (Figure 5.9).

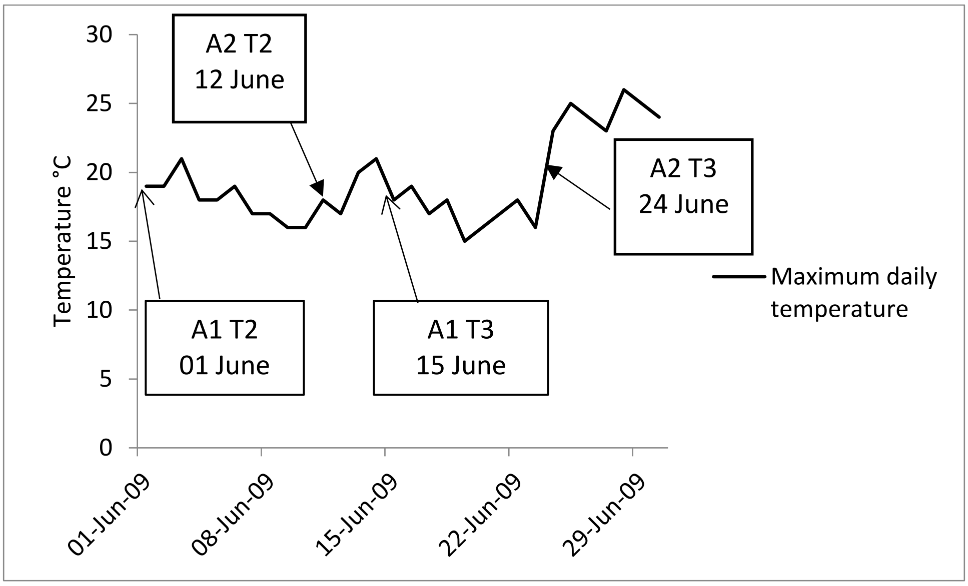


Figure 5.9: Maximum daily temperature at Lydden in June 2009. Arrows indicate spray applications – application 1 (A1) for treatment 2, 01 June at five flowering racemes and application 2 (A2), 12 June, application 1 (A1) for treatment 3, 15 June at first pod formation and application 2 (A2), 24 June.

Descriptive statistics and box plot for damage recorded on bulk subsamples taken from the trial at Tilney St. Lawrence in 2011 are shown in Table 5.13 and Figure 5.10. Maximum daily temperature is shown for 2011 in Figure 5.11. Descriptive statistics and box plot for damage recorded on bulk subsamples taken from the trial at Tilney St. Lawrence in 2012 are shown in Table 5.14 and Figure 5.12. Maximum daily temperature is shown for 2012 in Figure 5.13.

Table 5.13: Descriptive statistics for mean *B. rufimanus* damaged grains per treatment, recorded as proportionate weight, at Tilney St. Lawrence in 2011, with mean total weight of grains per subsample and mean weight of damaged grains per subsample for each treatment. 4 bulk subsamples were assessed per treatment. Coefficient of Variation is given for each treatment.

Descriptive statistics					95% confidence interval for mean				
Treatment	N	Mean proportion damaged grain	Standard deviation	Standard Error	Lower bound	Upper bound	Min	Max	CV%
Untreated	4	0.1515	0.0377	0.0188	0.0916	0.2114	0.1227	0.2037	24.85
1st pod + 13 days (without temperature threshold)	4	0.0779	0.0217	0.0108	0.0434	0.1124	0.0491	0.0955	27.85
1st pod + 13 days (with temperature threshold)	4	0.0816	0.0078	0.0039	0.0692	0.0939	0.0742	0.0917	9.52
1st pod + 13 days + 8 days (with temperature threshold)	4	0.0815	0.0179	0.0089	0.0530	0.1099	0.0580	0.0998	21.94

Treatment	Mean weight of grain per subsample (g)	Mean weight of damaged grain per subsample (g)
Untreated	1122.10	170.55
1st pod + 13 days (without temperature threshold)	1354.23	104.55
1st pod + 13 days (with temperature threshold)	1530.88	123.23
1st pod + 13 days + 8 days (with temperature threshold)	1456.45	117.55

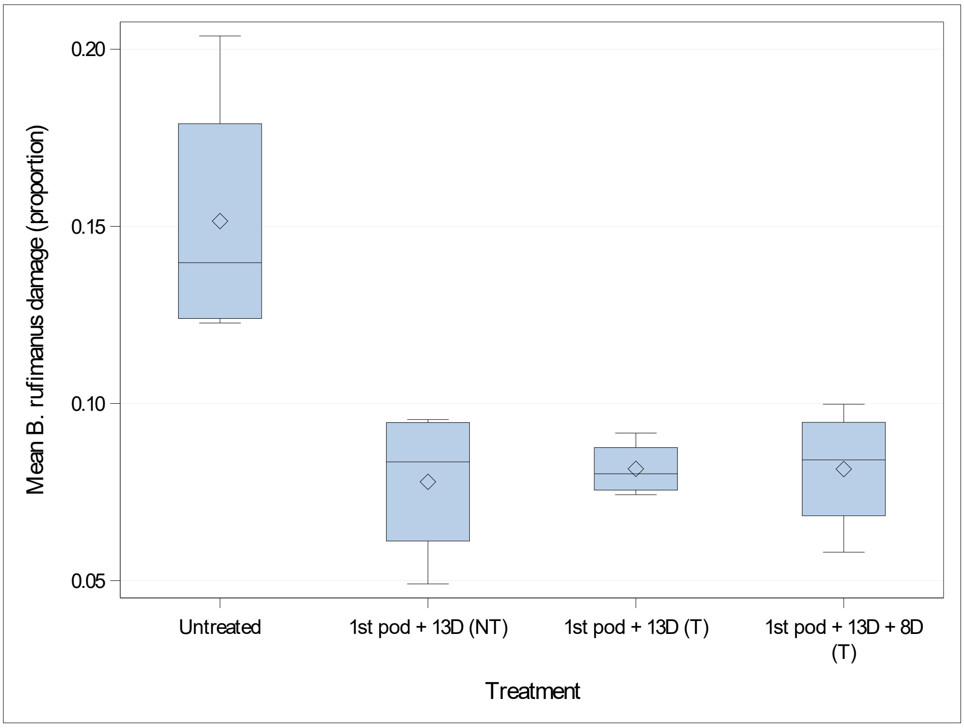


Figure 5.10: Mean *B. rufimanus* damage per treatment, recorded as proportionate weight, at Tilney St. Lawrence in 2011. Number of observations per treatment is 4 bulk subsamples. Whiskers represent maximum and minimum values. NT = no temperature threshold require, T = 2 consecutive days at 20°C required prior to application.

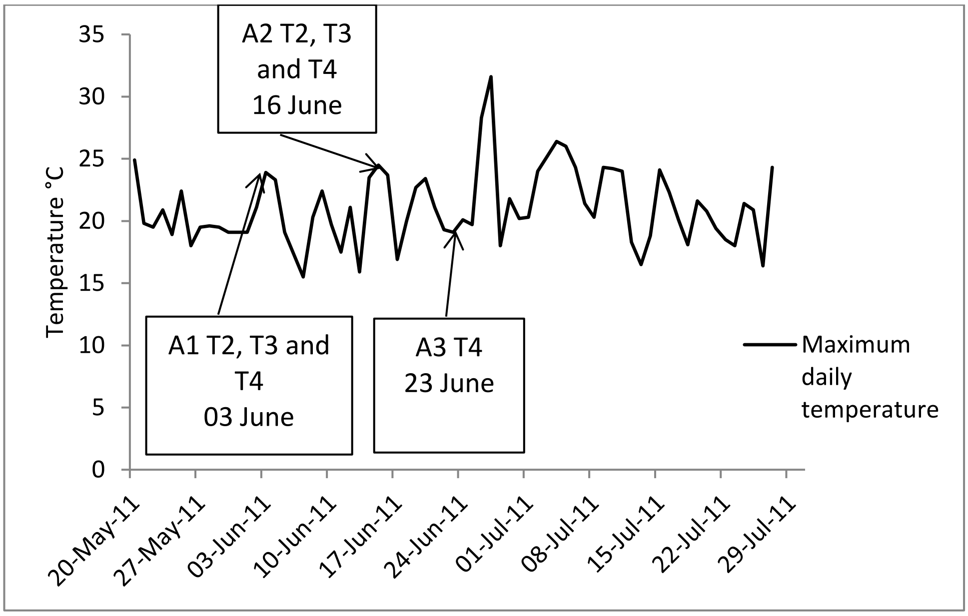


Figure 5.11: Maximum daily temperature at Tilney St. Lawrence in May, June and July 2011. Arrows indicate spray applications – application 1 (A1) for treatments 2, 3 and 4, 03 June at first pod formation, application 2 (A2) for treatments 2, 3 and 4, 16 June and A3 for treatment 4, 23 June.

The proportion of damage recorded on the bulk samples collected from the untreated plot at Tilney St. Lawrence in 2011 was higher on than all of the treated plots (Table 5.13; Figure 5.10) but the treated plots appeared to have similar proportions of damaged grains. Temperature had reached 20°C for two consecutive days when the first applications were made to treatments 2, 3 and 4 (Figure 5.11). This exactly coincided with first pod, leading to applications to all treatments on the same day, whether the temperature threshold was required or not.

There were no clear differences in the proportion of damage between treatments at Tilney St. Lawrence in 2012, although treatment 2, in which two sprays were applied starting at 1st pod without requirement for a temperature threshold appeared to have a lower proportion of damage comapred to other plots (Table 5.14; Figure 5.12). The inclusion of a third application in treatment 4 provided no additional benefit and appeared to lead to an increase in the proportion of damage to grains. The formation of *V. faba* pods began on 10 June in 2012 but the first insecticide applications were delayed until 19 June due to difficult weather. Temperature did not reach 20°C for two consecutive days immediately preceding A1 applications for treatments two and three, only for single days on 19 and 23 June and on the day of application on 25 June (Figure 5.13). Temperature fluctuated and there were few extended warm periods.

Table 5.14: Descriptive statistics for mean *B. rufimanus* damaged grains per treatment, recorded as proportionate weight, at Tilney St. Lawrence in 2012, with mean total weight of grains per subsample and mean weight of damaged grains per subsample for each treatment. 4 bulk subsamples were assessed per treatment. Coefficient of Variation is given for each treatment.

Descriptive statistics					95% confidence interval for mean				
Treatment	N	Mean proportion damaged grain	Standard deviation	Standard Error	Lower bound	Upper bound	Min	Max	CV%
Untreated	4	0.0592	0.0167	0.0084	0.0326	0.0858	0.0421	0.0822	28.26
1st pod + 14 days (without temperature threshold)	4	0.0332	0.0075	0.0037	0.0213	0.0451	0.0250	0.0406	22.53
1st pod + 14 days (with temperature threshold)	4	0.0457	0.0156	0.0078	0.0208	0.0706	0.0244	0.0621	34.23
1st pod + 14 days + 15 days (with temperature threshold)	4	0.0612	0.0265	0.0132	0.0180	0.1023	0.0361	0.0960	44.01

Treatment	Mean weight of grain per subsample (g)	Mean weight of damaged grain per subsample (g)
Untreated	422.00	23.50
1st pod + 13 days (without temperature threshold)	340.00	11.45
1st pod + 13 days (with temperature threshold)	374.65	15.70
1st pod + 13 days + 8 days (with temperature threshold)	454.90	25.00

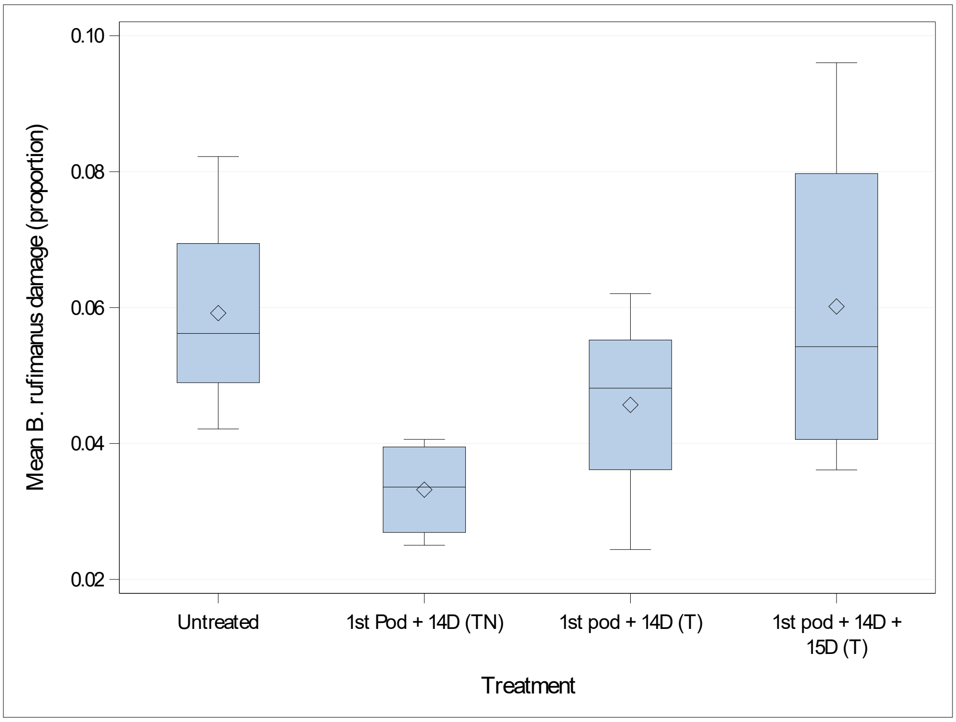


Figure 5.12: Mean *B. rufimanus* damage per treatment, recorded as proportionate weight, at Tilney St. Lawrence in 2012. Number of observations per treatment is 4 bulk subsamples. Whiskers represent maximum and minimum values.

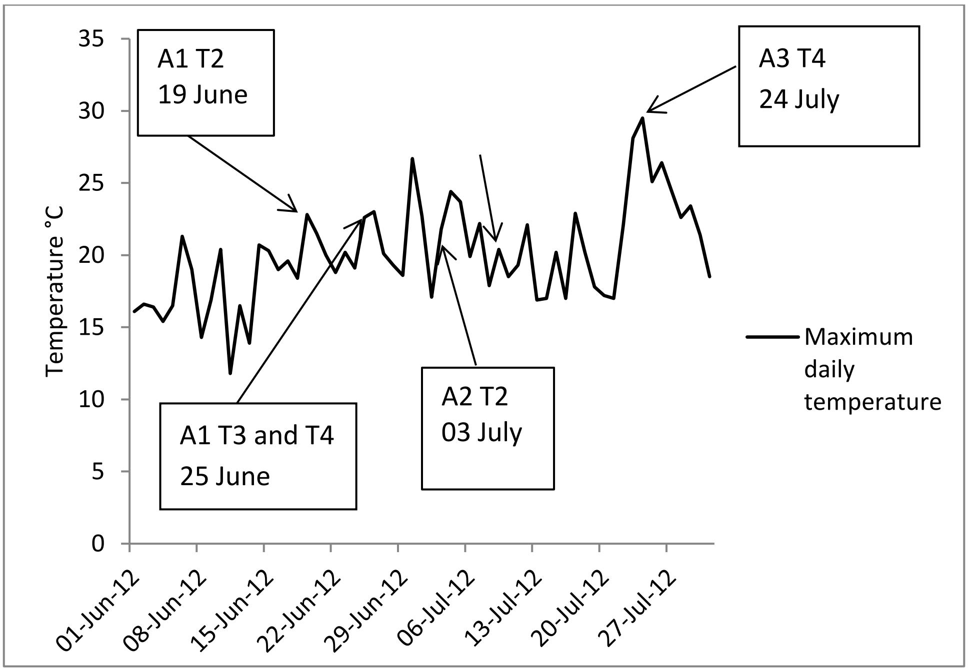


Figure 5.13: Maximum daily temperature at Tilney St. Lawrence in June and July 2012. Arrows indicate spray applications – application 1 (A1) for treatment 2, 19 June 10 days after first pod formation, application 1 (A1) for treatments 3 and 4, 25 June, application 2 (A2) for treatment 2, 03 July, application 2 (A2) for treatments 3 and 4, 09 July, application 3 (A3) treatment 4, 24 July.

5.3.2 *The influence of temperature on oviposition*

There were large differences in oviposition recorded between 2012, 2013 and 2014, in areas that had received no insecticide applications at Tilney St. Lawrence (Figure 5.14).

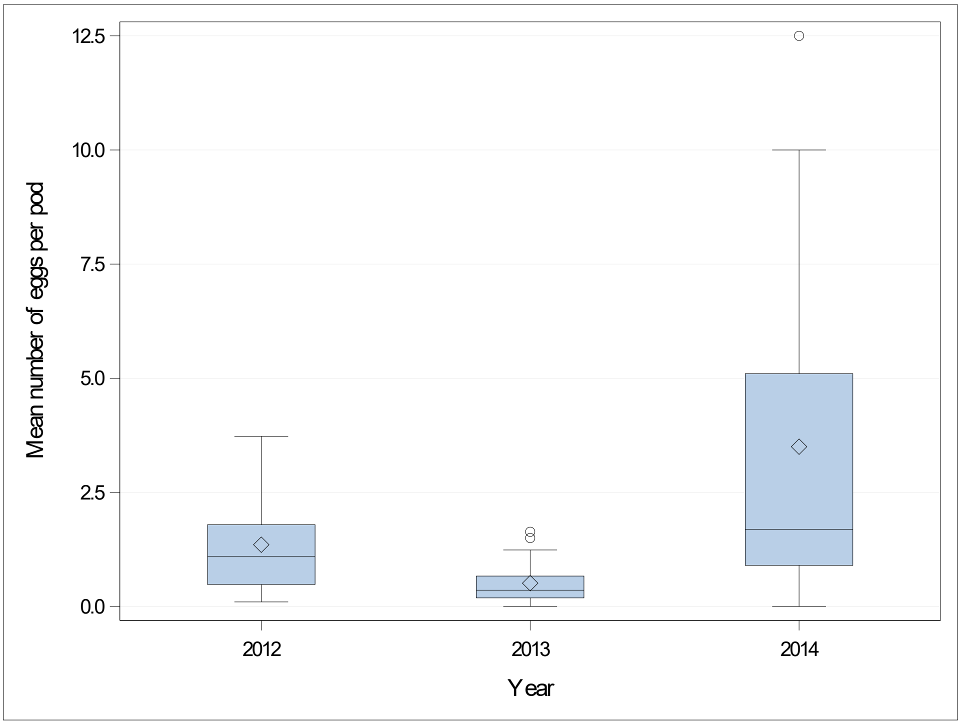


Figure 5.14: Mean number of *B. rufimanus* eggs per pod in each year, 2012, 2013 and 2014 on an unsprayed area of *V. faba* at Tilney St. Lawrence. Number of observations per year is 20 plants. Whiskers represent maximum and minimum values or 1.5 X Interquartile Range where suspected outliers are represented as unfilled circles.

Higher numbers of eggs were recorded in 2014 and this corresponds to the higher levels of damage recorded at the site in 2014 compared to 2012 and 2013 (Table 5.15). No statistical analyses were carried out to determine the relationships between number of eggs per pod and mean percentage damage as the number of observations for each year was small and comparisons could not be made between bulk samples of grains and plants assessed for numbers of eggs.

Table 5.15: Mean maximum daily temperature for June and July, date of first flower and pod formation and mean *B. rufimanus* damage, in samples collected from untreated areas of *V. faba* crop, recorded as percentage weight at Tilney St. Lawrence, 2012 to 2014.

Year	Mean maximum daily temperature °C		Date of emergence of *B. rufimanus* from over wintering	Date of first flower	Date of first pod set	Mean % *B. rufimanus* damage at Tilney St. Lawrence
	June	July				
2012	18.86	21.22	13-May	24-May	10-Jun	5.92
2013	19.69	25.36	21-May	03-Jun	21-Jun	3.96
2014	21.34	23.85	06-May	01-Jun	25-Jun	11.27

Descriptive statistics and analysis of between year difference in mean number of eggs per pod in untreated areas are shown in table 5.16. A Kruskal-Wallis test showed statistically significant differences between years. Variation in the number of eggs per pod for each year was high.

Table 5.16: Descriptive statistics and analysis of between year difference in number of eggs per pod at Tilney St. Lawrence using the Kruskal-Wallis Test, with pairwise comparison of years using the Dwass, Steel, Critchlow-Fligner Method.

Descriptive statistics					95% confidence interval				
Treatment	N	Mean number eggs per pod	Standard deviation	Std. Error	Lower bound	Upper bound	Min	Max	CV%
2012	20	1.3532	1.0826	0.2407	0.8466	1.8599	0.1000	3.7273	79.99
2013	20	0.5105	0.4728	0.1057	0.2893	0.7318	0.0000	1.6364	92.60
2014	20	3.5008	3.5885	0.8024	1.8213	5.1803	0.0000	12.5000	102.51
Kruskal-Wallis Test				Pairwise two-sided comparison analysis using Dwass, Steel, Critchlow-Fligner Method					
							DSCF Value	Pr > DSCF	
Chi-Square		19.0735		2012 vs. 2013			3.9800	0.0136	
DF		2		2012 vs. 2014			2.9460	0.0933	
Pr > Chi-Square		<.0001		2013 vs. 2014			5.7231	0.0002	

Mean maximum daily temperature during June in 2012 and 2013 was lower than that experienced in 2014 (Table 5.15, Figures 5.15, 5.16 and 5.17), and maximum daily temperature during the peak oviposition period in the two weeks following the formation of first pods was greater than 20°C for longer periods in 2014.

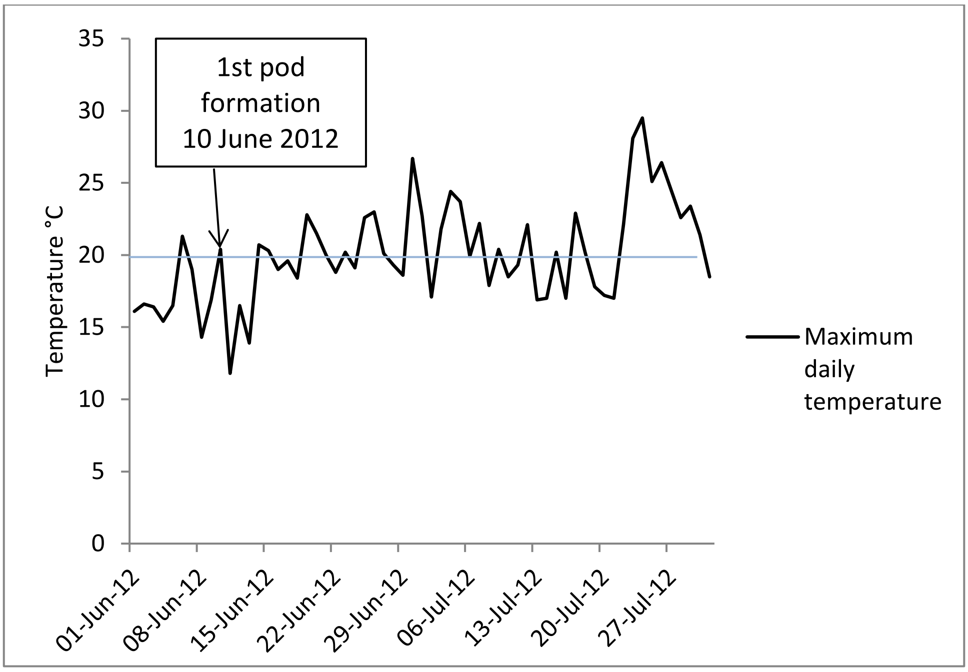


Figure 5.15: Maximum daily temperature at Tilney St. Lawrence in June and July 2012. Arrow indicates date of first pod formation (10 June).

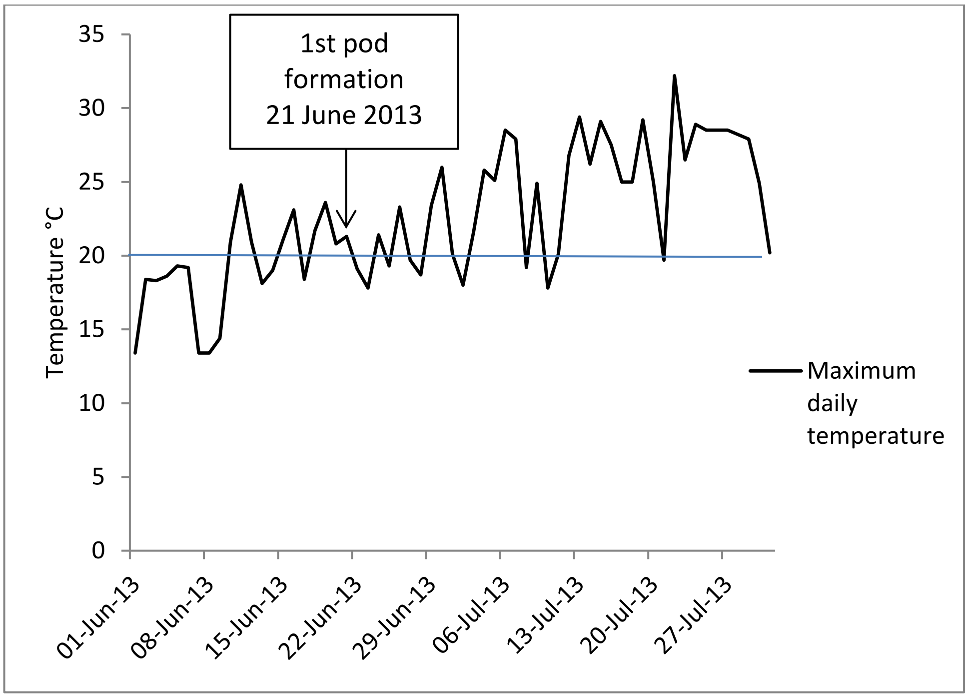


Figure 5.16: Maximum daily temperature at Tilney St. Lawrence in June and July 2013. Arrow indicates date of first pod formation (21 June).

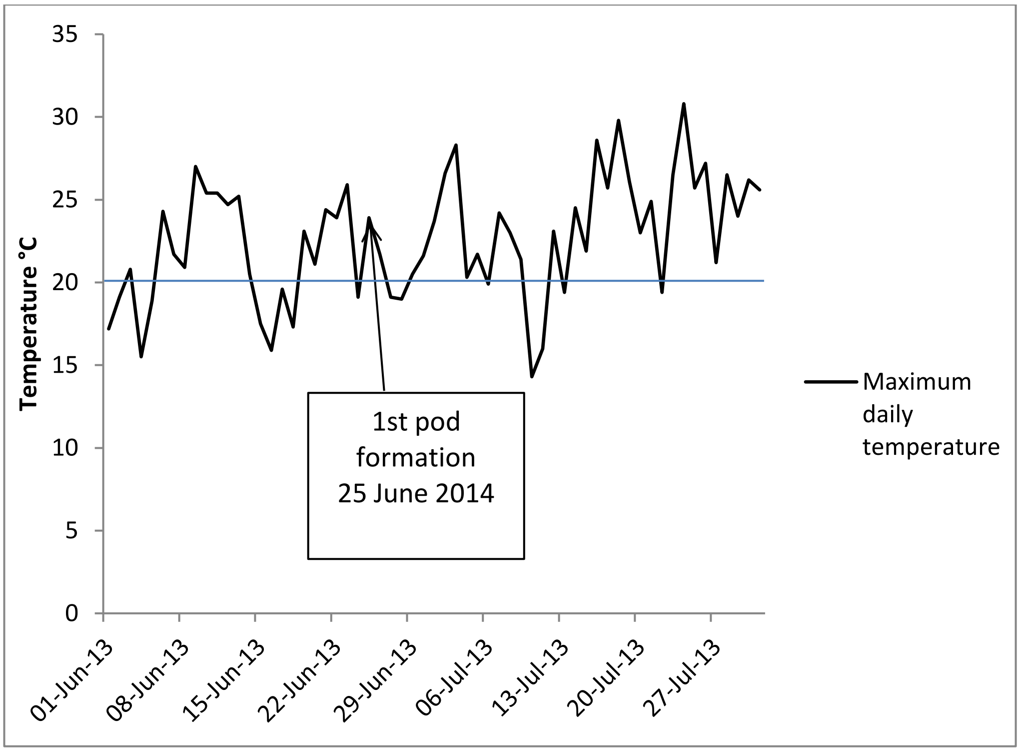


Figure 5.17: Maximum daily temperature at Tilney St. Lawrence during June and July 2014. Arrow indicates date of first pod formation (25 June).

5.4 Discussion

5.4.1 *Field trials to establish the effect of crop development, insecticide applications and temperature on activity and damage*

Treatments in an experiment designed to produce data suitable for statistical analysis should be isolated from each other in space or time. In a manipulative experiment interspersion of treatments results when experimental units are assigned to treatments by randomisation procedures. Randomisation is a way of achieving interspersion in a way that reduces the likelihood of bias and the risk of a type I error. For preliminary assessment of the suitability of experimental designs, interspersion may be a more practical criterion than randomisation (Hurlbert, 1984). It is not possible to know the probability of a type I error if there is lack of independence of errors, and interpretation of statistical analyses becomes subjective.

When undertaking field-scale trials on-farm however, it may not be practical to design trials that are fully replicated and randomised, as discussed in Section 5.1. For several reasons, including the degree of mobility of *B. rufimanus* and the use of farm equipment to carry out operations, large plots were selected to carry out observations in this study. This has caused a degree of uncertainty when evaluating results, and it may be more appropriate to consider the results presented here as observations or visual demonstration of effects, while aiming to infer as much as possible from the information generated. Added uncertainty may arise from farm operations that are not within the control of the observer, such as timing of applications or the collection of subsamples. To mitigate the risk of errors in collection of subsamples, further data collection was undertaken in 2010 and 2013 on plants that were examined for proportion of damage immediately prior to harvest. In 2011 and 2012, weather affected the application of insecticides to the detriment of the experiments, and little can be concluded from trials carried out in 2011. The coincidence of first pod formation with the test threshold temperature, two consecutive days when maximum daily temperature reached 20°C, led to insecticide applications to all treatments being made on the same day. As such any difference between the treatments in 2011 may have been due to random factors, although the insecticide treatments produced very similar levels of damage, with an observed level of damage to the untreated plot that was higher than all treated plots (Table 5.13; Figure 5.10).

At Tilney St. Lawrence in 2009 the temperature reached the threshold of two consecutive days at 20°C prior to both of the first applications for treatments 2 and 3, but at Lydden the temperature threshold was reached only prior to application one for treatment 3, in which the first application was made at the formation of first pods (figures 5.7 and 5.9). There appeared to be no discernible effects of crop growth stage and temperature on effectiveness of insecticide control of *B. rufimanus*. The decision to apply first insecticides in trials in the following years at the formation of first pods was made with a practical and commercial consideration. Applications prior to the formation of first pods appeared to control *B. rufimanus* adults in *V. faba* crops. However, the period of oviposition of *B. rufimanus* has been shown to extend for up to 45 days in the UK with peak oviposition occurring during the first two weeks following formation of pods (Chapter 4). Adults are highly mobile and may reinvade crops a few days after insecticides have been applied. Given the availability of insecticides to control *B. rufimanus* in *V. faba*, limited to pyrethroids only, the minor difference observed between applications at early flowering and at the formation of first pods, and the necessity to reduce the impact of insecticide applications on beneficial organisms, including bees, hoverflies, parasitoid wasps and carabid beetles, first application at the formation of first pods presents a reasonable compromise for growers, reducing the number of applications while providing some improved control compared to no treatment.

The data from Tilney St. Lawrence in 2010 showed a little variability between the treated plots. Plant samples taken within the untreated plot had higher levels of damage than treated plots, but there were only small differences between treatments, and in fact the plot that received two applications (treatment 3) compared to one (treatments 2 and 4) had slightly higher levels of damage (Figure 5.1). From the bulk grain samples collected during mechanical harvest, a higher level of damage was recorded in the treatment in which the insecticide was applied 26 days after the formation of first pods compared to all other treatments, but the untreated plot showed no difference from plots in which insecticides were applied singly or twice from the time of first pod formation (Figure 5.2). Temperature prior to all applications was at least 20°C for two consecutive days. The relatively high levels of damage overall when compared to 2009 indicated higher levels of adult activity possibly due to greater average temperature in June and July.

The lack of difference between any treatments and the untreated plot at Tilney St. Lawrence in 2012 (Figure 5.12) may be explained by low temperature during June and July and periods of high rainfall in that period, causing difficulty applying insecticides at the targeted timings. All applications were delayed due to rainfall and there were very few periods when temperature reached or exceeded 20°C for more than one day during June. Only during the third week of July did temperature reach 20°C for more than two days. The East Anglian region experienced 187% average rainfall in June 2012 and 212% in July compared to the 30 year average (Met Office b, 2016). The plot that had three insecticide applications had overall higher levels of damage than the other plots.

In 2013 at Tilney St. Lawrence, the highest proportion of damage was observed on plants that had received two insecticide applications when a temperature threshold was reached, compared to no threshold and the untreated plot (Figure 5.4).

When examining the data overall, it is not possible to reach firm conclusions about the influence of temperature and spray applications on the proportion of damage in each plot. However, there is a trend that arises across all years that indicates that those plots that had two or three insecticide applications, particularly at the first pod growth stage and when a temperature threshold was reached, appeared to have higher levels of damage compared to the other treated plots, and sometimes compared to the untreated plot. This warrants further investigation and may indicate a negative effect of pyrethroid applications to beneficial insects within the crop canopy. In 2013 there was also an observed difference in the number of grains formed per plant, the untreated plot having more grains per plant than the treated plots (Table 5.9).

Although definite conclusions may not be drawn, it should be considered whether spray applications caused damage to pollinators and natural predators, leading to reduced pod set on treated plots and greater damage by *B. rufimanus*. Natural enemies contribute considerable value to agriculture by suppressing pests that attack crop plants and it is important to understand how agricultural practices influence natural enemy communities. While insecticides are used to manage crop pests, they also disrupt control of pests by natural enemies, leading to unexpected outbreaks of crop pests (Douglas and Tooker, 2016). Parasitism by Hymenoptera causes some mortality of *B. rufimanus* larvae (Ahmed, 1996). De Luca (1965) listed eight Braconidae, one Eurytomidae, five Pteromalidae and one Trichogramma species that have been found to parasitise *B. rufimanus* globally. *Triaspis luteipes* Thomson and *Dinarmus laticeps* Ashmead (a synonym of *Dinarmus basalis*

(Rondani)) were the only two from de Luca (1965) that were recorded as parasites of *B. rufimanus* in the UK. A species found in *V. faba* seeds in Cambridgeshire, UK in 1998 was identified as *T. luteipes*, from the Braconidae family (M. Shaw, 1999, Personal Communication). *Triaspis* species always attack the host early in its life, either while it is a young larva, or possibly at embryo stage in the egg, thus, in the field in the case of *T. luteipes* parasitising *B. rufimanus*. They are internal parasites of the host larva and the host continues to grow after colonisation, most likely being killed as a pre-pupa (M. Shaw, 1999, Personal Communication). Although it is thought that UK *B. rufimanus* populations are not suppressed to commercially acceptable levels by Hymenoptera parasites, despite high numbers being present in seeds in some years, it may be that natural enemies have more effect on *B. rufimanus* populations than previously known.

5.4.2 *The influence of temperature on oviposition*

The relationship between number of eggs per pod and mean percentage damage could be seen in 2012, 2013 and 2014, but no meaningful statistical analysis could be carried out to determine whether this was the case due to the lack of comparability between samples examined for presence of eggs and bulk samples taken at harvest. Work carried out in 1996 and 1997 showed a degree of variation in the relationship between the number of eggs per plant and the level of damage to grain on the same plants (Ward, 1999). Regression analysis of the study in 1996 showed a strong positive relationship between number of eggs per plant and the number of damaged seeds per node, but the same was not observed in 1997.

Other studies examine the effect of temperature on oviposition, showing in many instances reductions in oviposition when temperature is lower (Van Baaren, *et al.*, 2005; Hall *et al.*, 2011; Dembilio *et al.,* 2012). A relationship could be seen between June and July maximum daily temperature and the number of eggs per pod. It is possible that temperature in the two week period following the formation of first pods was an important influence on the level of oviposition. In 2014, temperature following formation of first pods was higher than that experienced in 2012 and 2013. The period of higher temperature following oviposition in 2014 was continuous after the first two days following the formation of pod set. In 2012 and 2013 maximum daily temperature reached 20°C following pod formation but for a maximum duration of two days at a time. It is possible that the period of continuous high temperature in the two weeks following pod formation and commencement of oviposition in 2014 led to greater levels of oviposition. Conclusions from field studies may be unsuitable to determine the effect of temperature on oviposition, and laboratory or glasshouse studies, undertaken under controlled conditions, would provide a more robust test of effects (Regniere, *et al.* 2012). Regniere *et al.* (2012) discussed the importance of the collection of accurate data on development rates, survival and reproduction at several temperatures, including extremes, considering it essential for predicting phenological changes in a fluctuating environment. Under field conditions in the UK, it is unlikely that extreme temperature events would be experienced to test temperature effects sufficiently to provide models of phenological development for *B. rufimanus*. Further work to examine the effect of temperature in controlled conditions would be desirable to determine more clearly the threshold at which oviposition starts. However, it is necessary to extrapolate controlled studies to field conditions, in which many factors affect the usefulness of models of insect development, such as variation in microclimate conditions, particularly in habitats with dense canopies. The degree of variation in temperature under field conditions may also affect insect responses, as may the quality of the host plant, which can be subject to much variability depending on soil conditions and weather (Zaugg, *et al.,* 2013). It has been shown that Mexican bean weevil, *Zabrotes subfasciatus* (Boheman), shows behavioural and physiological adaptation in oviposition behaviour according to host availability (Teixeira and Zucoloto, 2012). When beans were scarce and competition high, beetles laid more eggs onto the same seed and fewer, less fecund adults emerged. This may impact on beetle population densities when plant resources vary in their availability or quality from season to season.

From both the damage and oviposition studies, further work is recommended. An investigation should be undertaken in *V. faba* to determine the impact of pyrethroid insecticides on the population of beneficial insects. A further study of temperature requirements to stimulate oviposition should be undertaken in controlled conditions.

Chapter 6: The influence of sowing date, cultivar and plant density on the damage caused by *B. rufimanus* in *V. faba*

6.1 Introduction

Improvements or alterations to basic crop management practices such as sowing time, use of tolerant cultivars or plant spacing are often effective at reducing pest attack, even with relatively unsophisticated knowledge of the crop or pest, and can be introduced at different levels of agricultural development (FAO, 2017). Integrated Pest Management (IPM) is defined by the FAO (2017) as 'the careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimise risks to human health and the environment.' UK organisations, including Defra, the National Farmers Union and BASIS Registration Ltd. have adopted and promote the use of IPM in agriculture as a primary means to aid the reduction of chemical usage and improve farm management practices. The Sustainable Use of Pesticides Directive (European Parliament, 2009) created a framework for targets and measures to reduce risks and impacts of pesticide use on human health and the environment, and to encourage the development of IPM and alternative approaches to reduce dependency on the use of pesticides. The Directive made the implementation of the principles of integrated pest management obligatory, with priority given wherever possible to non-chemical methods of plant protection and pest and crop management.

Several strategies may be employed to reduce pest attacks to more acceptable levels, by making the environment less suitable for pest survival, dispersal, growth and reproduction (Hill, 1989). Strategies can include altering plant density, timing of sowing or the use of trap cropping. Stoddard *et al.* (2009) described management techniques including tillage, sowing date, plant density and effective weed control as methods with potential to reduce pest incursions.

Modification of sowing date can lead to the crop, or the crop resources most utilised by insect pests, such as flowers or pods, becoming unavailable to the pest during the period when attack is likely to be most severe, or during key life stages of the pest (Pedigo and Zeiss, 1995; Sastawa *et al.,* 2003; Bell and Crane, 2016). However, the influence of sowing date modification on crop yield and performance needs also to be considered.

Plant breeding for varietal tolerance to pests and diseases has several advantages over chemical control, reducing the quantity of harmful substances being used, leading to positive impacts for beneficial organisms, and reducing the likelihood of pesticide resistance arising in key crop pest species. The introduction of resistant or tolerant cultivars allows improvements in prevention and reduction of crop damage with little or no requirement for technical modifications to farm practices (Keneni *et al.,* 2011). Physiological characteristics of *V. faba* influence their susceptibility to attack by pests. Cultivar, day length and temperature affect time of flowering, and the number of flowers per node may vary with cultivar, plant density and weather. Timing of flowering or pod formation are important influencing factors where attack by *B. rufimanus* is expected, and this is linked to sowing timing and weather. To date there has been no recorded resistance or tolerance to *B. rufimanus* in commercial cultivars in the UK or France, although differences in susceptibility were observed between cultivars in Poland (Roubinet, 2016). Research is being undertaken to breed tolerant cultivars in the projects 'PeaMUST' (Burstin *et al*., 2017) and 'Development of genomic tools for marker-assisted selection (MAS) in faba bean and pea for resistance to faba bean weevil (*B. rufimanus*) and pea weevil (*B. pisorum*)' (INRA, 2018). The projects seek to identify tolerant genotypes, to identify the genes involved in the tolerance mechanisms and integrate these genotypes in breeding programs.

The use of plant density as a cultural control may cause yield benefits to outweigh the quality reduction caused by some insect pests, or affect plant growth in a way that leads to reduced impact by pests, either by causing an increase in the population of natural enemies, or by providing an environment that repels the insect pest. For some pests, an improvement in crop vigour caused by altering plant density, can lead to the impact of attack being reduced.

Crop development and cultivar affect the activity of *B. rufimanus* and the quantity of damage caused in *V. faba* crops (Szafirowska, 2012). Work undertaken in Syria (Tahhan and van Emden, 1989) showed that cultivars with late flowering and pod formation characteristics showed phenotypic resistance to *Bruchus dentipes* Baudi (Coleoptera: Bruchidae). Medjdoub-Bensaad *et al*. (2007) also documented the synchronisation of *B. rufimanus* reproduction with the flowering and fruiting phases of the host plant.

There are current approvals in *V. faba* in the UK for pyrethroid insecticides, and a neonicotinoid substance, thiacloprid, gained approval for use in *V. faba* in November 2017 (HSE, 2018) to control *B. rufimanus*. Field beans in the UK receive up to three applications of pyrethroid insecticides during the period of peak activity of *B. rufimanus*, from mid-flowering and early pod formation until the later pod-filling stage. There is a risk that these insecticide applications may pose a risk to non-target species such as pollinators. There is considerable variation in the level of autofertility of different cultivars of *V. faba* (Marcellos and Perryman, 1990) and discussion regarding the contribution of insect pollination to yields (Stoddard, 1986; Suso *et al*., 1996), but it is generally considered that pollination from bees and beneficial insects provides between 30 and 60% of the pollination requirement of *V. faba* and in some cases more (Delaplane and Mayer, 2000; Cunningham and Le Feuvre, 2013). Disruption to bee activity through pesticide use, including foraging behaviour, motor function, grooming and wing fan behaviour, as a result of insecticide use, is now well documented (Vandame and Belzunces, 1998; Zhou *et al*., 2011; Gill and Raine, 2014; Sanchez-Bayo and Goka, 2014; Oliver *et al*., 2015). Growers make applications either early in the morning or late in the evening to avoid foraging bees and other beneficial insects during flowering, and many prefer not to apply insecticides during this period. There are no effective products approved for use in organic systems to control the pest (FERA, 2016), although evidence is available for control of *B. rufimanus* using entomopathogenic microorganisms (Sabbour and E-Abd-El-Aziz, 2007).

The aim of this study was to develop alternative strategies for *B. rufimanus* control to help reduce the impact of insecticides on beneficial organisms, with the hypothesis that sowing date influences the degree of *B. rufimanus* damage in *V. faba* crops in the UK. Additional aims were to determine the effects of *V. faba* cultivar and plant density on *B. rufimanus* activity and damage, and to investigate the effects of the three factors on *V. faba* yield. Trials were established within the project 'Improving the availability of UK sourced protein feed through new faba bean varieties, production and utilisation systems (Optibean)' (co-funded by Innovate UK, PGRO, Wherry and Sons Ltd., NIAB-TAG, Garford Farm Machinery Ltd. and the Waitrose producer groups, with Aberystwyth University as the academic partner). The study of *B. rufimanus* damage to *V. faba* was not an objective of the project and work carried out to investigate the effect of sowing date, cultivar and plant density on *B. rufimanus* damage was not funded by the project.

6.2 Methods

Spring sown *V. faba* trials were established at three sites in Lincolnshire during March and April 2015. The sites were at Stubton (Grid reference SK885488), Lincoln (Grid reference TF019744) and Dowsby (Grid reference TF154291). The experimental treatments consisted of two sowing dates at Stubton and Lincoln, and three sowing dates at Dowsby (Table 6.1). Two commercial spring bean cultivars, Fury and Fuego, were planted at four sowing densities, 20, 40, 60 and 80 plants per square metre. The three factors were randomized in blocks and arranged in a split plot design, with the sowing dates in main blocks, the cultivar as sub-plots within the main blocks and the plant density as sub plots of cultivar. Each sub-sub plot (10m x 1.75m; plant density) was replicated three times (Plate 6.1).

Table 6.1: *V. faba* sowing dates at three sites in East Anglia, Stubton, Dowsby and Lincoln, evaluated for the effect of sowing date, cultivar and plant density on *B. rufimanus* damage in 2015.

Site	**Sowing date 1**	**Sowing date 2**	**Sowing date 3**
Stubton (Grid Reference SK885488)	06-March-15		07-April-15
Lincoln (Grid Reference TF019744)	11-March-15		10-April-15
Dowsby (Grid Reference TF154291)	11-March-15	27-March-15	10-April-15

Sowing date blocks were separated by a different cultivar of *V. faba* as a discard area. Plots were treated with standard herbicides and fungicides to prevent crop failure, and two insecticides were applied to control pea and bean weevil (*Sitona lineatus* L.) at an early crop growth stage. No insecticides were applied during flowering and pod formation to control *B. rufimanus*.

Plate 6.1: *V. faba* trial design showing the layout of plots at three sites in East Anglia, to evaluate the effects of sowing date, cultivar and plant density on *B. rufimanus* damage in 2015.

Plots were harvested using a Wintersteiger trials combine harvester and harvest dates are shown in Table 6.2. Plot yield and grain moisture content were recorded, and yield per plot standardised to 15% moisture content using the formula: Yield (at 15% grain moisture) = Grain yield × (100 – actual grain moisture %)/85. Grain samples weighing approximately 400 grams were taken from three replicates of each treatment at Stubton and Lincoln and two replicates of each treatment at Dowsby and examined and classified for damage caused by *B. rufimanus*, either as damaged or undamaged beans. Damage was recorded as percentage weight of damaged beans. Symptoms of damage are described in Chapter 5, Section 5.2.1.

Table 6.2: Harvest dates for three sites in East Anglia, Stubton, Dowsby and Lincoln, in 2015.

Site	Harvest date
Stubton	09-September-15
Lincoln	17-September-15
Dowsby	10-September-15

The number of sowing dates was not evenly balanced across the three sites, but they were combined in a single analysis by having three levels of the factor ‘sowing date’ (1, 2 and 3). There were data available for all three sowing dates at Dowsby, but for Stubton and

Lincoln there were data for only two sowing dates (1 and 3) (Table 6.1). Sowing date 2 data for Stubton and Lincoln were entered into a mixed effects model as missing values.

Prior to analysis, data groups were checked for normality and homogeneity of variances using the Anderson Darling and Bartletts Tests, respectively. Where log transformation was used for mixed effects modelling for damage to grains, data presented graphically or in tables display the means and corresponding standard deviations derived from back-transformed data. Data for yield were not transformed.

All data were analysed using SAS® University Edition. Data for *B. rufimanus* damage were analysed using a mixed effects model with sowing date, cultivar and plant density as fixed effects and site as a random effect. Data for yield were analysed for each site individually using a mixed effects model with sowing date, cultivar and plant density as fixed effects, and for all sites in combination using a mixed effects model with sowing date, cultivar and plant density as fixed effects, and site as a random effect. Model simplification was used to remove terms that did not contribute significantly to the explanatory power of the model. Pairwise comparison of means, where significant effects were found, were made by Differences of Least Squares Means, adjusted using the Tukey-Kramer method. 95% confidence levels were used for all data analysis.

6.3 Results

6.3.1 ***Multi-site, multi-factor analysis of influence of sowing date, cultivar and plant density on damage***

A mixed effects model identified sowing date, cultivar and plant density as significant factors influencing percentage *B. rufimanus* damage to grain samples, with no statistically significant interactions between fixed effects (Table 6.3).

Table 6.3: Combined influence of sowing date, cultivar and plant density on damage caused to *V. faba* grain by *B. rufimanus* at Stubton, Lincoln and Dowsby in 2015, with site as a random effect. Stubton: Sowing 1 = 06 March 2015, sowing 3 = 07 April 2015; Lincoln: Sowing 1 = 11 March 2015, sowing 3 = 10 April 2015; Dowsby: Sowing 1 = 11 March 2015, sowing 2 = 27 March 2015, sowing 3 = 10 April 2015. Cultivars are Fuego and Fury, plant densities are 20, 40, 60 and 80 plants per m². N = 144. Data were Log-transformed for analysis.

Source	Sum of Squares	Mean Square	DF	Error DF	F Value	Pr > F
Sowing date	21.3959	10.6979	2	135	207.68	<.0001
Cultivar	3.2872	3.2872	1	135	63.82	<.0001
Plant density	0.6950	0.2317	3	135	4.50	0.0048
Site (random effect)	19.3796	9.6898	2	135	188.11	<.0001
Residual	6.9540	0.0515				
Total			143			

Table 6.4: Pairwise mean comparison of damage caused to *V. faba* grain by *B. rufimanus* at Stubton, Lincoln and Dowsby in 2015 for sowing date, cultivar and plant density, with site as a random effect. Stubton: Sowing 1 = 06 March 2015, sowing 3 = 07 April 2015; Lincoln: Sowing 1 = 11 March 2015, sowing 3 = 10 April 2015; Dowsby: Sowing 1 = 11 March 2015, sowing 2 = 27 March 2015, sowing 3 = 10 April 2015. Cultivars are Fuego and Fury, plant densities are 20, 40, 60 and 80 plants per m². N = 144.

Pairwise Mean comparison of sowing date, cultivar and plant density (with site as random effect)
Difference of Least Squares Means Test adjusted using the Tukey–Kramer method

Critical Value		2.0076		
Variable: Sowing date				
	N	Mean % damage	Group*	Standard Deviation
Sowing date 1	64	35.3308	a	18.1880
Sowing date 2	16	19.2538	b	5.0162
Sowing date 3	64	15.2691	c	6.9091
Variable: Cultivar				
Fuego	72	27.8396	a	16.8360
Fury	72	21.4167	b	15.0190
Variable: Plant density				
20	36	21.1284	c	12.2746
40	36	24.3651	b	15.5605
60	36	25.9251	ab	16.4915
80	36	27.0941	a	19.7381
Variable: Site (random effect)				
Stubton	48	16.5331	b	18.8691
Lincoln	48	38.4823	a	38.4823
Dowsby	48	18.8691	b	7.2802

*Means within a column with the same letter are not significantly different (p>0.05)

A significant difference in *B. rufimanus* damage was found between sowing dates when data from all sites were combined, all sowing dates having significantly different levels of damage compared to each other, with the highest percentage damage at sowing date 1 and the lowest at sowing date 3 (Table 6.4, Figure 6.1). The cultivar Fuego had a significantly higher percentage damage than Fury (Table 6.4, Figure 6.2). Plant density influenced damage across the sites, and damage at plant density of 20 plants per m² was significantly lower than that at 60 and 80 plants per m² (Table 6.4, Figure 6.3). There was a significant difference between sites, and higher levels of damage were seen at Lincoln compared to Stubton and Dowsby (Table 6.4). It is not known why this should be the case, although surrounding landscape features and density of cropping in neighbouring fields may contribute to the differences.

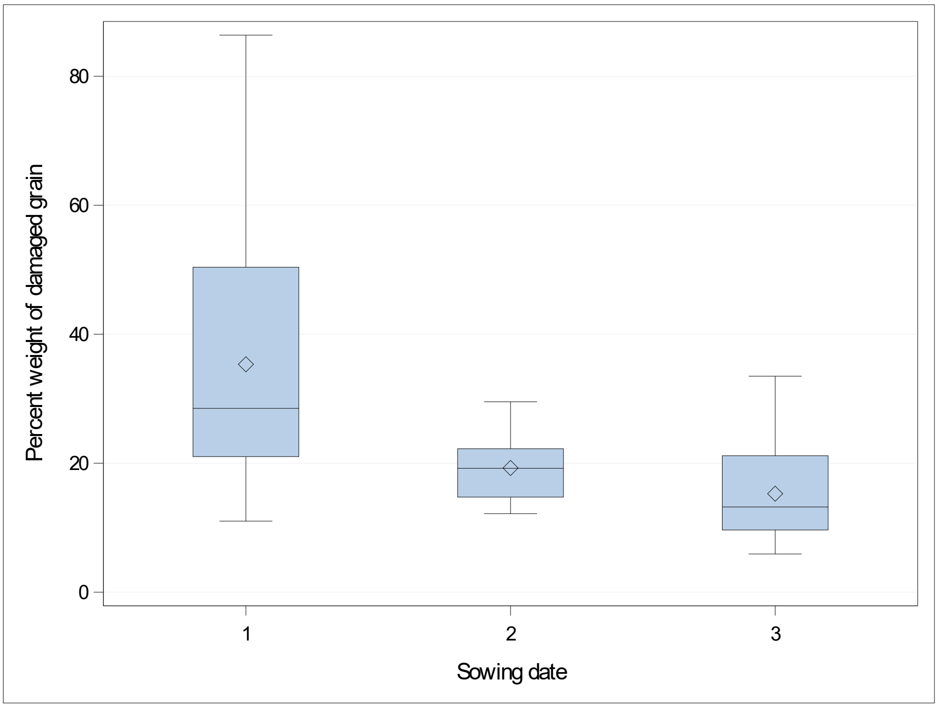


Figure 6.1: Mean *B. rufimanus* damaged grains per sowing date, recorded as percentage weight of grains, at Stubton, Lincoln and Dowsby in 2015. Number of observations is 144 grain samples. Boxes represent the interquartile range and whiskers represent minimum and maximum values.

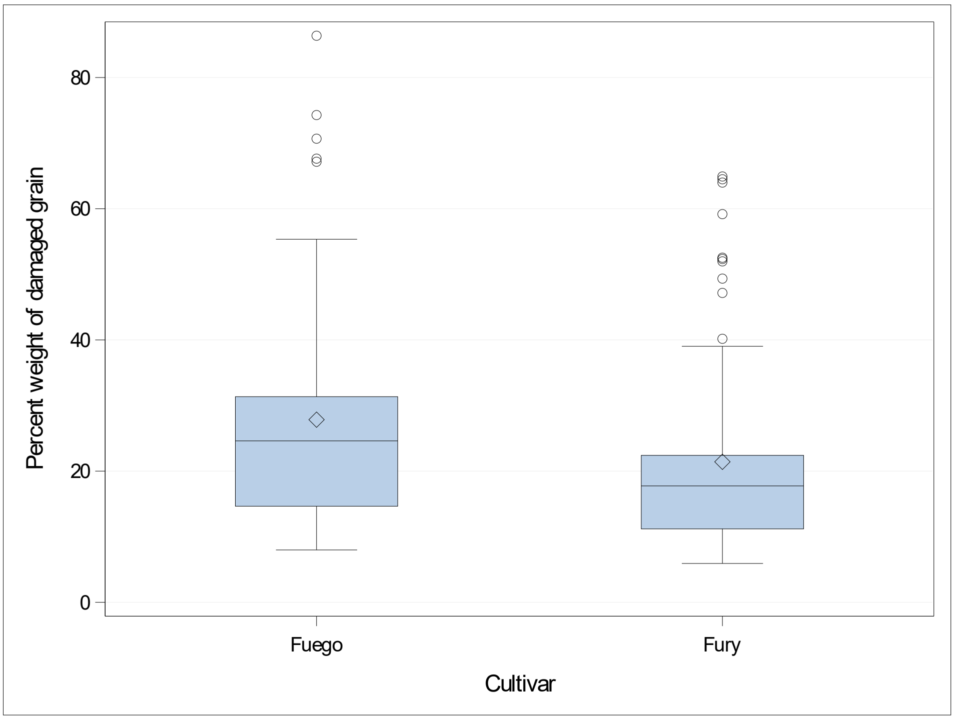


Figure 6.2: Mean *B. rufimanus* damaged grains per cultivar, recorded as percentage weight of grains, at Stubton, Lincoln and Dowsby in 2015. Number of observations is 144 grain samples. Boxes represent the interquartile range and whiskers represent minimum values and 1.5 X interquartile iange where suspected outliers are represented as unfilled circles.

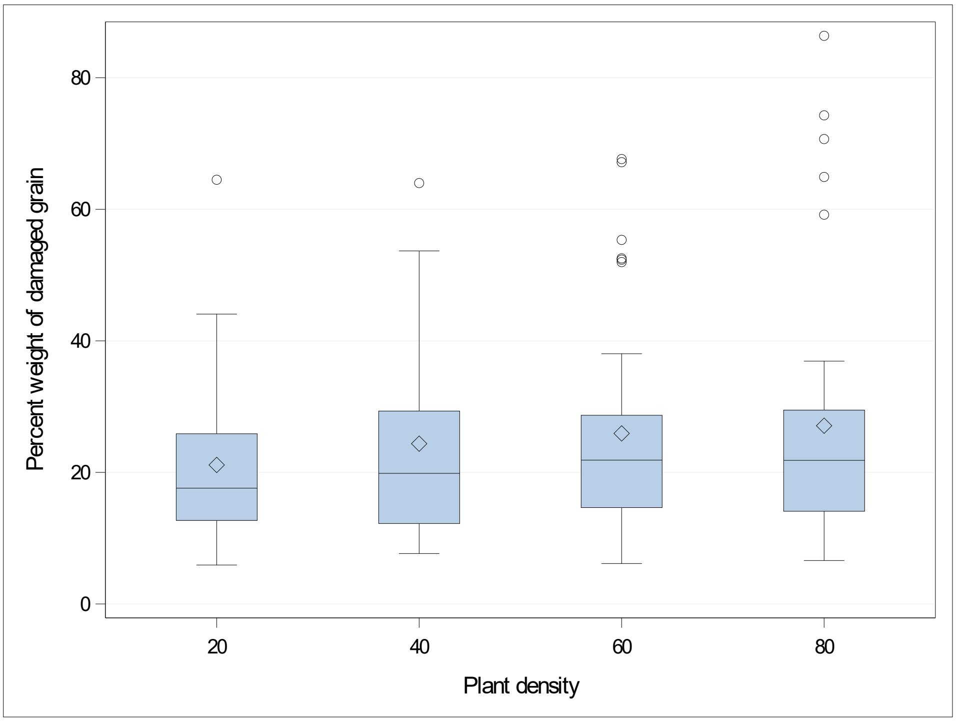


Figure 6.3: Mean *B. rufimanus* damaged grains per plant density, recorded as percentage weight of grains, at Stubton, Lincoln and Dowsby in 2015. Number of observations is 144 grain samples. Boxes represent the interquartile range and whiskers represent minimum values and 1.5 X interquartile range where suspected outliers are represented as unfilled circles.

6.3.2 *The influence of sowing date, cultivar and plant density on V. faba yield*

Data for yield were analysed for each site individually due to high yields experienced at Dowsby in 2015. Although a mixed effects model combining all sites showed that sowing date was a significant factor affecting yield, the between-site differences were not sufficiently explained (Appendix C, Tables XII and XIII). It is likely that soil type differences between sites influenced yield, and yields were much higher at Dowsby. At the Lincoln site soil type was shallow and lime-rich overlaying limestone, at Stubton, freely draining lime-rich loam, and at Dowsby, loamy, clay soils with naturally high groundwater (Natural England, 2017). The water-retentive soil-type at Dowsby may have caused yield to be greater than at Stubton or Lincoln, where soils were free-draining. The results in 2015 showed that there was a general suppression of yield when sowings were carried out at the later date in early April at Stubton and Dowsby (Figures 6.4 and 6.6).

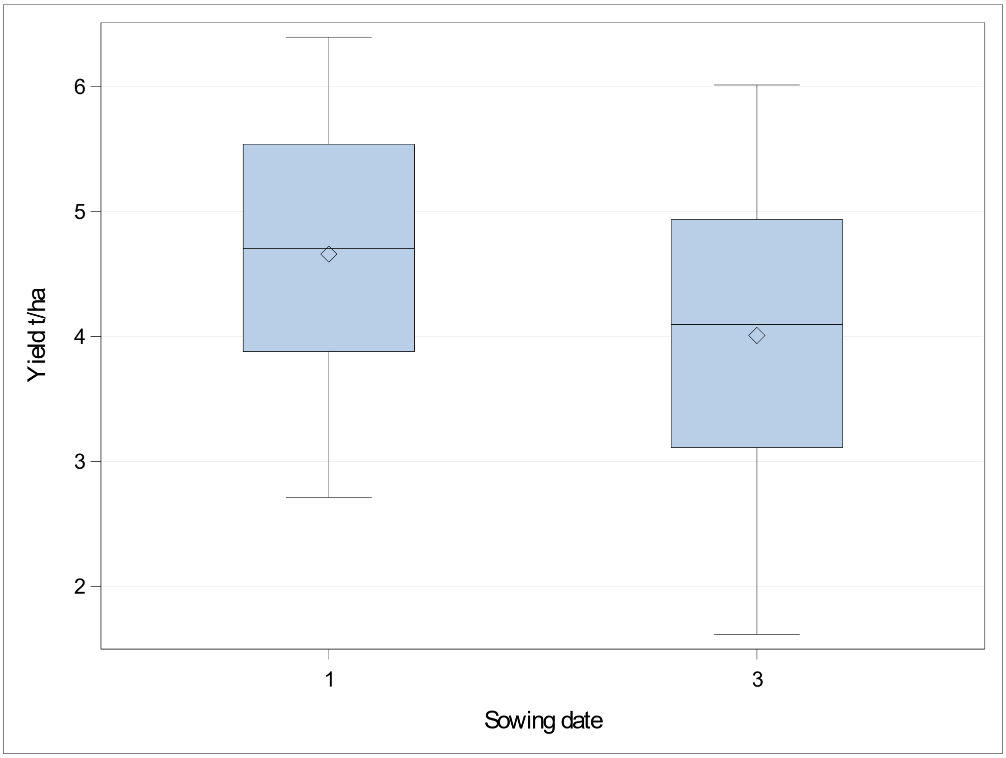


Figure 6.4: Mean yield of *V. faba* (tons per hectare) for each sowing date at Stubton in 2015. Sowing 1 = 06 March 2015, Sowing 3 = 07 April 2015. Sowing date was replicated 24 times. Boxes represent the interquartile range and whiskers represent minimum and maximum values.

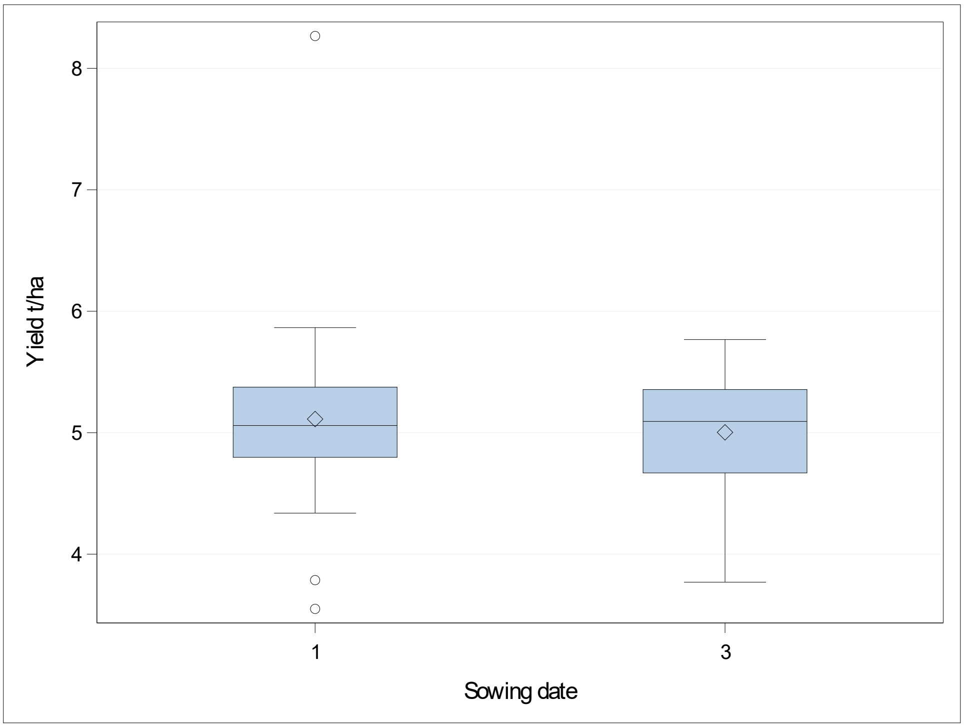


Figure 6.5: Mean yield of *V. faba* (tons per hectare) for each sowing date at Lincoln in 2015. Sowing 1 = 1 March 2015, Sowing 3 = 10 April 2015. Sowing date was replicated 24 times. Boxes represent the interquartile range and whiskers represent minimum and maximum values.

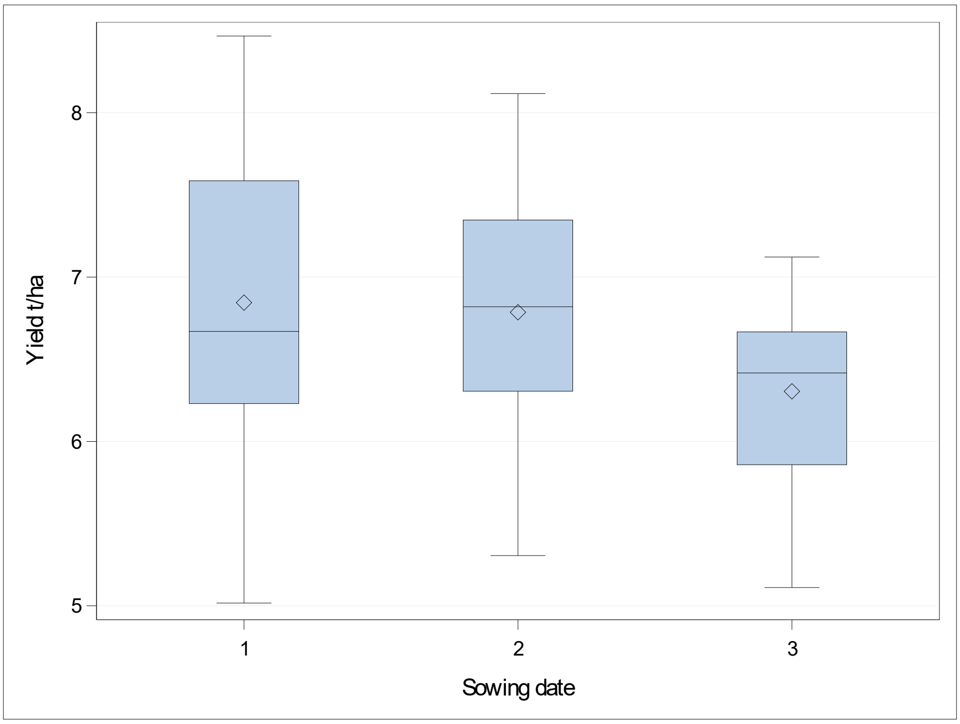


Figure 6.6: Mean yield of *V. faba* (tons per hectare) for each sowing date at Dowsby in 2015. Sowing 1 = 11 March 2015, Sowing 2 = 27 March 2015, Sowing 3 = 10 April 2015. Sowing date was replicated 24 times. Boxes represent the interquartile range and whiskers represent minimum and maximum values.

Table 6.5: Combined influence of sowing date, cultivar and plant density on yield (tons per hectare) at Stubton in 2015. Sowing 1 = 06 March 2015, sowing 2 = 07 April 2015, cultivars are Fuego and Fury, plant densities are 20, 40, 60 and 80 plants per m². N = 48.

Descriptive and summary statistics						
Variable	N Obs	Min	Max	Mean	St Dev	CV %
Yield (t/ha)	48	1.61	6.39	4.33	1.17	10.02
Response variable Yield t/ha						
Source	DF	Sum of squares		F Value	Pr (>F)	
Sowing	1	5.0895		18.28	0.0002	
Cultivar	1	16.7693		60.21	<.0001	
Density	3	31.0380		37.15	<.0001	
Cultivar* Density	3	0.6776		0.81	0.4972	
Sowing* Cultivar	1	1.4033		5.04	0.0318	
Sowing* Density	3	0.3571		0.43	0.7347	
Sowing* Cultivar* Density	3	0.2578		0.31	0.8190	
Pooled Error	21	8.9117				
Total	47	64.5043				

Table 6.6: Pairwise mean comparison of yield at Stubton in 2015 for sowing date and cultivar combination, with pairwise mean comparison of density. Sowing 1 = 06 March 2015, sowing 2 = 07 April 2015, cultivars are Fuego and Fury, plant densities are 20, 40, 60 and 80 plants per m^2. N = 48.

Variable: Yield (t/ha) Difference of Least Squares Means Test adjusted using the Tukey–Kramer method			
Comparison of Sowing at each level of Cultivar			Critical value 0.4711
Sowing date	N	Mean yield (t/ha)* Cultivar Fuego	Mean yield (t/ha)* Cultivar Fury
1	24	4.2385 a	5.0786 a
2	24	3.2452 b	4.7693 a
Comparison of Cultivar at each level of Sowing			Critical value 0.4711
Cultivar	N	Mean yield (t/ha)* Sowing 1	Mean yield (t/ha)* Sowing 2
Fuego	24	4.2385 b	3.2452 b
Fury	24	5.0786 a	4.7693 a
Pairwise Mean Comparison of Density			Critical value 0.8149
Plant density	N	Mean yield (t/ha)*	
20	12	3.11 d	
40	12	4.23 c	
60	12	4.67 bc	
80	12	5.32 a	

*Means within a column with the same letter are not significantly different $p>0.05$

Sowing date, plant density and cultivar significantly affected yield at Stubton, as did the combination of sowing and cultivar (Table 6.5). No significant difference in yield was found when examining the combinations cultivar and density, sowing and density, or sowing, cultivar and density. Yield of Fuego at sowing 2 was significantly lower than at sowing 1 (Table 6.6). There were no significant differences in yield of Fury between sowing dates. Fuego had significantly lower yield than Fury at both sowing dates. Plant density had a significant effect on yield, and as plant density increased, so did yield.

Table 6.7: Combined influence of sowing date, cultivar and plant density on yield at Lincoln in 2015, with pairwise mean comparison of plant density. Sowing 1 = 11 March 2015, sowing 2 = 10 April 2015, cultivars are Fuego and Fury, plant densities are 20, 40, 60 and 80 plants per m^2. N = 48.

Descriptive and summary statistics

Variable	N Obs	Min	Max	Mean	StDev	CV %
Yield (t/ha)	48	3.55	8.27	5.06	0.7070	11.57

Response variable Yield (t/ha)

Source	DF	Sum of square	F Value	Pr (>F)
Sowing	1	0.1436	0.42	0.5228
Cultivar	1	1.2849	3.74	0.0622
Density	3	7.9537	7.71	0.0005
Cultivar* Density	3	0.8739	0.85	0.4786
Sowing* Cultivar	1	0.7099	2.06	0.1606
Sowing* Density	3	0.1881	0.18	0.9077
Sowing* Cultivar* Density	3	1.3292	1.29	0.2953
Pooled Error	21	11.0085		
Total	47	23.4918		

Variable: Yield (t/ha)
Difference of Least Squares Means Test
adjusted using the Tukey–Kramer method

Critical Value 0.8149

Pairwise Mean Comparison of Density

Plant density	N	Mean yield (t/ha)*
20	12	4.37 b
40	12	5.17 a
60	12	5.28 a
80	12	5.41 a

*Means within a column with the same letter are not significantly different (p>0.05)

Sowing date and cultivar did not significantly affect yield at Lincoln, although plant density did (Table 6.7). There were no significant interactions between factors that influenced yield. When plant density was 20 plants/m^2, yield was significantly reduced compared to all other plant densities.

Table 6.8: Combined influence of sowing date, cultivar and plant density on yield at Dowsby in 2015. Sowing 1 = 11 March 2015, sowing 2 = 27 March 2015, sowing 3 = 10 April 2015, cultivars are Fuego and Fury, plant densities are 20, 40, 60 and 80 plants per m². N = 72.

Descriptive and summary statistics						
Variable	N Obs	Min	Max	Mean	StDev	CV %
Yield (t/ha)	72	5.02	8.47	6.65	0.8054	9.60
Response variable Yield (t/ha)						
Source	DF	Sum of square		F Value	Pr (>F)	
Sowing	2	4.2115		5.17	0.0092	
Cultivar	1	1.6705		4.10	0.0484	
Density	3	16.8397		13.79	<.0001	
Cultivar* Density	3	1.3151		1.08	0.3678	
Sowing* Cultivar	2	1.0043		1.23	0.3003	
Sowing* Density	6	0.7466		0.31	0.9309	
Sowing* Cultivar* Density	6	0.7317		0.30	0.9341	
Pooled Error	48	19.5378				
Total	71	46.0571				

Table 6.9: Pairwise mean comparison of sowing date, cultivar and plant density at Dowsby in 2015. Sowing 1 = 11 March 2015, sowing 2 = 27 March 2015, sowing 3 = 10 April 2015, cultivars are Fuego and Fury, plant densities are 20, 40, 60 and 80 plants per m². N = 72.

Variable: Yield (t/ha) Difference of Least Squares Means Test adjusted using the Tukey–Kramer method		
Pairwise Mean Comparison of Sowing		
Critical Value		0.7033
Sowing date		Mean yield (t/ha)*
Sowing	N	
1	24	6.84 a
2	24	6.79 a
3	24	6.30 b
Pairwise Mean Comparison of Cultivar		
Critical Value		0.4619
Cultivar	N	Mean yield (t/ha)*
Fuego	36	6.49 b
Fury	36	6.80 a
Pairwise Mean Comparison of Density		
Critical Value		0.8000
Plant density	N	Mean yield (t/ha)*
20	18	5.83 b
40	18	6.78 a
60	18	6.88 a
80	18	7.09 a

*Means within a column with the same letter are not significantly different ($p>0.05$)

Sowing date, cultivar and plant density significantly affected yield at Dowsby (Table 6.8). Sowing 3 had significantly lower yield than sowings 1 and 2 (Table 6.9). Fuego had significantly lower yield than Fury. When plant density was 20 plants/m², yield was significantly reduced compared to all other plant densities (Table 6.9).

6.4 Discussion

6.4.1 *The influence of sowing date on damage*

Sowing date influenced damage to *V. faba* grain by *B. rufimanus*, and there were statistically significant differences in *B. rufimanus* damage between all the sowing dates, a reduction in damage seen as sowing occurred later (Tables 6.3 and 6.4, Figure 6.1). Spring field beans are usually sown from the beginning to the end of March in the UK (PGRO, 2016). In difficult conditions, when weather prevents seedbed preparation, they may be sown until mid-April. As demonstrated by analysis of the influence of sowing date on yield (Sub-section 6.3.2), later sowing may lead to a risk of reduced yields. The additional risk may arise that harvesting may be delayed until October when crop and grain drying conditions become more difficult. These risks are discussed further in Sub-section 6.4.3.

Later sowing leads to later flowering and pod set, which are critical growth stages for the reproductive development and oviposition activity of female *B. rufimanus*. Since the termination of reproductive diapause for the species is dependent on specific stimuli (photoperiod, temperature and food source), reproductive diapause does not end, particularly for females, if flowers are not present. The mobility of *B. rufimanus* is high and beetles are able to fly from crop to crop to coincide with variable flowering periods. Oviposition by *B. rufimanus* occurs only on the pods of species of *Vicia* and *Lathyrus* and females must wait for pod development before oviposition commences (Huignard *et al.*, 1990; Johnson and Romero, 2004; Delobel and Delobel, 2006).

National and local data shows that autumn sown *V. faba* consistently has higher levels of damage, possibly due to earlier flowering and pod formation (Frontier Agriculture Ltd., 2015, Personal Communication; Chapter 3). Flower bud formation in autumn sown *V. faba* cultivars may be up to five weeks earlier than in spring sown cultivars in some years, and pod formation up to four weeks earlier. The data from this study indicated that later sowing of spring sown *V. faba* strongly influenced the level of *B. rufimanus* damage caused to grain. The period of peak oviposition occurs during the first two weeks following the end of diapause and may last one to two months depending on region and climate (Hamani and Medjdou-Bensaad, 2015) with a gradual decline in oviposition towards the end of that period (Chapter 4). It is possible that when pods were formed later they were less exposed to periods of high oviposition that occurred soon after female reproductive diapause ended.

Damage from *B. rufimanus* is higher in the southern and eastern areas of the UK (Chapter 3) and growers aiming for the premium, human-consumption, export market to Egypt and North Africa often have difficulty reaching quality standards required for this market. The use of sowing date to provide an alternate means of reducing *B. rufimanus* damage would allow growers in some regions of the UK to reduce insecticide use during *V. faba* flowering and early pod formation stages, thus reducing input costs and preventing further damage to pollinating insects. In some regions, particularly areas where damage caused by *B. rufimanus* is lower and more sporadic, it is possible that this strategy may help growers to eliminate applications of pyrethroid insecticides for *B. rufimanus* control.

6.4.2 *The influence of cultivar and plant density on damage*

Mixed effects models showed that cultivar and plant density had statistically significant effects on damage caused to *V. faba* grain by *B. rufimanus* (Tables 6.3 and 6.4, Figures 6.2 and 6.3). The cultivar Fuego had higher levels of grain damage than Fury (Figure 6.2). Plant density led to significant differences in percentage *B. rufimanus* damage, and increased plant density caused higher levels of damage (Table 6.4). *V. faba* plots at plant density of 20 plants per m² had significantly lower levels of damage than those planted at 40, 60 and 80 plants per m².

In this study, cultivar had an important influence on the level of damage caused by *B. rufimanus*. UK *V. faba* cultivars are described each year in the Recommended List for Pulse Varieties (PGRO, 2016). Characteristics are rated on a 1 to 9 scale for each cultivar, with rating 9 indicating that the cultivar shows the character to a high degree. Characteristics include yield, flower colour, earliness of ripening, shortness of straw, standing ability at harvest, resistance to disease, thousand seed weight and protein content, although agronomic and phenotype differences are small between UK cultivars. Earliness of ripening has remained similar for the cultivars Fuego and Fury for several years at a rating of seven, with a small change to rating eight for Fury in 2016 (PGRO, 2016). This character is measured shortly before harvest and may not reflect more subtle differences in timing of flowering and pod formation. It is possible that timing of pod formation varied between the two cultivars, leading to variation in damage caused by *B. rufimanus*, although this was not observed within this study and is unlikely given the small differences between UK cultivars. There is published evidence of the influence of cultivar on damage caused to *V. faba* grain by *B. rufimanus* (Ebedah *et al*., 2006; Szafirowska, 2012), although the mechanism is not well understood, and only phenotypical resistance is likely to exist. It is likely that plant architecture, flowering period and abundance, and the timing of pod formation are the key factors that influence activity of *B. rufimanus*. More recent research identified pod wall and seed coat resistance of *Pisum sativum* to *Bruchus pisorum* (Aryamanesh *et al*., 2014).

Although plant density influenced damage at the three sites in this study there are difficulties in explaining this. There is evidence that *V. faba* floral semiochemicals are very attractive to *B. rufimanus* (Bruce *et al*., 2011) and it may be expected that increased flower density, for instance when planting density increases, would be more attractive to *B. rufimanus*, leading to greater damage, as seen in combined analysis of the three sites in this study (Table 6.4). However, it is also possible that reduced pod density occurs when planting density is reduced, potentially leading to proportionately higher levels of oviposition on a fewer number of pods. The commercial optimum plant density for spring sown *V. faba* is 55 plants per square metre. It is unlikely that any major benefits would be achieved by reducing plant density to manage damage caused by *B. rufimanus*, as cropping changes may impact directly and detrimentally on yield and crop value to growers (Sub-section 6.4.3).

6.4.3 *The influence of sowing date, cultivar and plant density on V. faba yield*

Sowing date, cultivar and plant density significantly affected yield at Stubton and Dowsby (Tables 6.5 and 6.8). Yield was lower when *V. faba* was sown in early to mid-April compared to early March and Fuego produced significantly lower yield than Fury at Stubton and Dowsby. Sowing date and cultivar did not significantly affect yield at Lincoln, although plant density did (Table 6.7). Overall, yield increased as plant density increased, and yield was significantly suppressed when *V. faba* was planted at 20 plants per m^2 at all sites (Tables 6.6, 6.7 and 6.9).

The general suppression of *V. faba* yield when sown later at all sites may in part be explained by shortfalls of rainfall following sowing. *V. faba* is a species that does not tolerate dry conditions well and yield is suppressed when insufficient water is available (Sprent *et al.,* 1977; PGRO, 2016). Rainfall data for 2015 (Met Office b, 2016) indicated that February rainfall for East Anglia was 99% of the 30 year average (1981 to 2010) at 39.5 mm, potentially providing sufficient soil moisture for good establishment of *V. faba* planted in early March. March 2015 rainfall in East Anglia was just 58% of the 30 year average, at 25.1 mm, and April 2015 rainfall was only 49% of the 30 year average rainfall figure at 21.8 mm, although May rainfall was approximately the same as the thirty year average at 105%, 51.3mm. It is possible that soil moisture deficit in March and April caused yield to be reduced at the later sowing date in April at the three sites. Data from trials carried out between 2011 and 2015 in spring sown *V. faba* (PGRO, 2015) indicated that average yields declined when sowing was delayed until the first two weeks of April. The trend for declining yield in later sown *V. faba* would have financial impact for growers. A yield loss of 0.25 tons per hectare represents a financial loss of approximately £35 per hectare at 2016 grain trading prices (Farmers Weekly, 2016), and the data from 2015 indicated that yield loss was between 0.25 and 0.99 tons per hectare when sowing occurred in early to mid-April. This would represent a loss of up to £140 per hectare at 2016 prices. Although the use of later sowings may be useful to reduce *B. rufimanus* damage in some areas, particularly those that are on the margins of the lower levels of damage in the north of England, the risk of yield loss should be balanced against the benefit gained from premium prices received for improved quality. The value of the crop varies from year to year and therefore it is important that growers re-evaluate prices and risks annually. For those growers that wish to reduce pyrethroid insecticides applications, and in organic systems, management of sowing date may provide a substantial benefit for the control of *B. rufimanus*.

The multi-site analysis carried out using a mixed-effects model showed that sowing date, cultivar and plant density were significant factors affecting yield (Appendix C, Tables XII and XIII). The later sown *V. faba* plots had significantly lower yield than the earlier sown plots, although the influence of high yields at Dowsby, the only site at which sowing 2 was present, led to more difficulty interpreting the results. The analysis of cultivar difference showed that Fury had a higher yield and Fuego, and analysis of plant density showed that 20 plants per m^2 had significantly lower yield than all other plant densities.

Chapter 7: Observations of *B. rufimanus* overwintering habitats and pollen feeding behaviour prior to overwintering

7.1 Introduction

Many insect species choose protected overwinter sites to provide protection against low temperatures (Danks, 2006). Sheltered habitats reduce the risk of ice damage and slow the rate of temperature change. Survival of some species may be possible only after very slow cooling (Miller, 1978). The choice of overwintering sites is therefore very important. Adult *B. rufimanus* enters over-wintering sites during September and October following emergence from *V. faba* grain in the UK, and is in reproductive diapause, although active, at this time. It is possible that *B. rufimanus* undergoes one of the mechanisms described by Kostal (2006) (Chapter 2), a very gradual decrease in metabolic rate accompanied by behavioural and physiological activities required to prepare for overwintering, such as intense feeding prior to seeking a suitable microhabitat. Danks (2002) discussed the functions of insect dormancy periods, such as energy conservation, prevention of development during inappropriate periods, protection from adverse conditions and synchronisation with the host plant as well as the types of dormancy that favour survival in conditions of varying climate and habitat suitability. Tran *et al.* (1993) demonstrated that a period of at least three months of continuous cold and darkness was required before *B. rufimanus* became responsive to factors that would terminate reproductive diapause. If the period of exposure to these conditions was less than three months, reproductive diapause termination was suppressed in both males and females, although the processes were complex and variation existed within the study. A period of cold temperature below 10°C was particularly important for the conservation of metabolic reserves.

Studies were undertaken between 2009 and 2013 to identify the habitats that hosted *B. rufimanus* during the winter period and to investigate factors that influenced survival during the winter. Anecdotal evidence exists for the presence of *B. rufimanus* in habitats containing flowering plants in the autumn, such as garden hedgerows and flowering field margins, the possibility being that these provide valuable food sources prior to overwintering. If true, this may indicate possibilities for management of *B. rufimanus* as it enters overwintering sites, such as trapping or catch-cropping, or weed control measures that may reduce the availability of food resources prior to overwintering. Several habitats were studied including hedgerow base vegetation, grass and flowering field margins of arable fields, grassland and woodland edges. Sampling techniques such as trapping, hand searching, vacuum sampling and soil coring were used to determine the most commonly used sites for over-wintering. Natural England (D. Sheppard, 2010, Personal Communication) provided advice about invertebrate survey techniques for hedgerows and woodland trees, including the use of habitat surrogates, bark removal and baited, interception and aerial pitfall traps. Factors such as vegetation structure and available food sources during the autumn following adult emergence from the crop were recorded. *B. rufimanus* mortality rates were investigated using artificially created winter habitats, and by studying survival of adults following sowing of *V. faba* seed in which adults had overwintered. The studies described here should be considered observational, although parts of the study were carried out using more systematic sampling techniques. *B. rufimanus* may survive in the seed over winter, and there is some evidence that they survive the process of planting in more arid climates (Medjdoub-Bensaad *et al.,* 2007). A small experiment was carried out to test whether this is likely in the UK.

7.2 Methods

7.2.1 *Observations of feeding behaviour prior to overwintering*

Observations were made of the feeding behaviour of *B. rufimanus* in non-host plant species in field margins surrounding a commercial crop of spring sown *V. faba* following the end of *V. faba* flowering at Bourne, Lincolnshire (Grid reference TF086213) on 17 July 2009. The plant species were recorded, and images collected to provide evidence of feeding behaviour. A systematic survey was not carried out.

7.2.2 *Suction sampling and turf sampling*

Suction and turf sampling techniques were selected for sampling grassland and ground vegetation for the presence of *B. rufimanus,* based on evidence of the effectiveness of these methods for the collection of invertebrates in grassland habitats (Brook *et al.,* 2008). Sampling was undertaken at Crowland, Lincolnshire (Grid reference TF268138) and Barnwell, Northamptonshire (Grid reference SP970849) and sites selected based on long histories of *V. faba* cropping within the rotation and reported history of *B. rufimanus* presence in crops and in *V. faba* grain, to optimise the likelihood of presence in overwintering habitats. Samples were collected from four habitats, three grassland habitats and one hedgerow base habitat, in January 2009 as follows: Crowland: A field corner managed within the existing Environmental Stewardship Scheme for wildlife benefit, including provision of habitat suitable for invertebrates and ground-nesting birds (DEFRA, 2005) (Plate 7.1). The area consisted of tussocky grass species and was managed using no pesticides or fertilisers and with no cutting, other than to reduce woody growth; A field margin adjacent to a ditch and containing tussocky grass species with some flowering plants and managed in the same way with no pesticide or fertiliser inputs and minimal cutting (Plate 7.2); Barnwell: The basal vegetation and leaf litter of a hedgerow adjacent to a farm track surrounding an arable field (Plate 7.3). The hedgerow was evaluated for type using a standard UK hedgerow survey form (Appendix D) (DEFRA, 2007); A grass field margin adjacent to a woodland edge and ditch and consisting of tussocky grass species (Plate 7.4). All habitats surveyed were located adjacent to fields in which *V. faba* was grown in 2008.

Plate 7.1: Survey area of grassland managed under Environmental Stewardship rules (DEFRA, 2005) for field corner management at Crowland, January 2009.

Plate 7.2: Survey area of grass field margin managed under Environmental Stewardship rules (DEFRA, 2005) adjacent to a ditch at Crowland, January 2009.

Plate 7.3: Survey area of hedgerow adjacent to a farm track at Barnwell, January 2009.

Plate 7.4: Survey area of grass field margin managed under Environmental Stewardship rules (DEFRA, 2005) adjacent to ditch and woodland edge at Barnwell, January 2009.

Samples were taken using a leaf vacuum shredder, modified to include a small-gauge sieve in the aperture of the vacuum to collect samples. The aperture was 150mm diameter. Samples were collected by running the vacuum at high power and pressing the aperture into the vegetation for 20 seconds at 30 random locations within each sampling site (Plate 7.5), producing a total of 120 samples from the four sites. Samples were placed into polythene bags and frozen for a short period prior to examination for the presence of *B. rufimanus*. Other invertebrate species were not recorded.

Plate 7.5: Vortis suction sampling in a grass field margin at Crowland in January 2009.

Plate 7.6: Soil sampling in a grass field margin at Crowland in January 2009.

Further samples were taken from each location using a soil core with an aperture of 100 millimetres diameter to a depth of approximately 60 millimetres to include the top layer of soil and vegetative growth (Plate 7.6). Each area was divided into 70 metre lengths from which three subsample cores were extracted and bulked to provide a single sample. A total of nine subsamples (three bulk samples) were collected from each habitat location, producing a total of 36 subsamples (12 bulk samples) from the four sites. Samples were placed into polythene bags and stored in cool conditions for a short period until examination. Samples were washed through brass sieves and the material contained by a 420µm gauge mesh retained for examination for the presence of *B. rufimanus*. Other invertebrate species were not recorded.

Four monitoring traps containing plant semiochemical lures were placed at both Crowland and Barnwell on 31 March 2009 to record emergence of *B. rufimanus* from overwintering sites and to verify the presence of *B. rufimanus* at each location. A description of methods for monitoring using semiochemical monitoring traps can be found in Chapter 4. Sward height was measured at each sampling point for each habitat.

7.2.3 *Artificial habitats – mesocosms and overwintering sleeves*

Two artificial mesocosms were constructed to determine the survival rate of *B. rufimanus* in a habitat consisting of grassland and leaf litter. The structures were located at Thornhaugh, Cambridgeshire (Grid reference TF071009) in an area of grassland adjacent to a small area of woodland. The mesocosms were constructed from short sections of plastic tube placed upright into the ground to a depth of 10cm (Plate 7.7) and placed in position on 13 October 2009. Captive populations of *B. rufimanus* that had emerged from *V. faba* seed following harvest in 2009 were maintained for a period of time at low temperature (4°C) in a standard domestic fridge, in plastic boxes covered with gauze and containing cotton wool soaked in a 10% sucrose solution. They remained under these conditions from the date of collection until placed inside the artificial habitats, a period of approximately four weeks. Advice about storage and maintenance of live adult *B. rufimanus* was obtained from entomologists at Rothamsted Research (Smart, 2009, Personal Communication). Leaf litter was placed into the artificial mesocosms and 100 live captive adult *B. rufimanus* placed into each one. Cotton wool soaked in 10% sucrose solution as a potential food source was placed into one of the mesocosms. Each plastic tube was covered with gauze to prevent adults escaping and a plastic cover erected to prevent flooding of the mesocosms. The artificial structures remained in place for the duration of the winter and emergence traps were placed over the structures in early spring 2010 prior to the emergence of *B. rufimanus* and following removal of the gauze and plastic cover, to determine adult survival. The emergence traps were constructed from metal rings measuring 0.78 metres diameter, placed in the ground to a depth of between five and 10cm, with struts over which netting was placed and a circular capped tube at the apex in which adult *B. rufimanus* emerging from the turf layer and leaf litter would be trapped. Plate 7.8 illustrates the design of emergence traps.

Plate 7.7: Artificial mesocosms to evaluate the survival of *B. rufimanus* in a grassland habitat in autumn 2009 at Thornhaugh, Cambridgeshire.

Plate 7.8: Design of emergence traps used to capture adult *B. rufimanus* on emergence from artificial mesocosms and placed on a potential overwintering site at Crowland, Lincolnshire in 2010.

Four insect overwintering sleeves were placed in hedge plants at Thornhaugh on 16 December 2010 (Plate 7.9). Captive populations of *B. rufimanus* that had emerged from *V. faba* grain following harvest in 2010 were maintained for a period at low temperature (4°C) in a standard domestic fridge, in plastic boxes covered with gauze and containing cotton wool soaked in a 10% sucrose solution. They remained under these conditions from the date of collection until placed inside the overwintering sleeves, a period of approximately 10 weeks. Overwintering sleeves were secured to hedge branches using rope ties fastened at each end of the sleeve to prevent escape of *B. rufimanus* during the period of the study. No additional food source was provided for the duration of the study. *B. rufimanus* populations were recorded prior to placement in the sleeves and sex determined. Overwintering sleeve 1 contained 50 adult *B. rufimanus* of mixed sex and the proportion of males to females was unknown; overwintering sleeve 2 contained 50 adult male *B. rufimanus*; overwintering sleeve 3 contained 50 adult female *B. rufimanus*; overwintering sleeve 4 contained 25 male and 25 female adult *B. rufimanus*. The sleeves were labelled at the time of placement to ensure accurate identification of samples in spring 2011. 50 captive adults from the overwintering sleeves, 25 male and 25 female, were frozen and dissected following removal from sleeves in spring 2011, to investigate reproductive development. Naturally established populations of adult *B. rufimanus* were captured using traps baited with floral semiochemical attractants as they emerged from overwintering sites at Tilney St. Lawrence

(Grid reference TF566144) in April 2011. 25 male and 25 female adults were frozen and dissected to investigate reproductive development. The reproductive organs of both males and females were examined, using a high-powered Leica® microscope with camera attachment. *V. faba* crop growth stage was monitored at Aylmer Hall to determine the date of onset of flowering in 2011.

Plate 7.9: Over-winter sleeve placed in a hedgerow at Thornhaugh, Cambridgeshire in December 2010 to evaluate winter mortality of *B. rufimanus*.

7.2.4 *Emergence traps*

Emergence traps were placed at Crowland, Lincolnshire (Grid reference TF270135) on 16 March 2010. The emergence traps were of the same construction as those used at Thornhaugh and three traps were placed on the tussocky grass field margin adjacent to the field in which spring sown *V. faba* was grown in 2009 (Plate 7.8) to capture adults, if present, as they emerged from turf during the spring. Four monitoring traps containing floral semiochemical attractants were placed at the same location to confirm the presence of *B. rufimanus* at the site. Temperature was recorded using a Tinytag® data logger contained within a Stevenson screen. Emergence and monitoring traps were inspected at regular intervals from 19 March 2010.

7.2.5 *Hedgerow, woodland edge and tree surveys*

Following anecdotal information about the presence of adult *B. rufimanus* in well-established standing trees, observations were made of *B. rufimanus* overwintering under the bark of trees at one site at Gedney Hill, Peterborough (Grid reference TF335109) in February 2011.

Hedgerow, woodland edge and tree surveys were carried out in February 2013 at locations adjacent to fields in which *V. faba* was grown in 2012. A hedgerow was surveyed at Tilney St. Lawrence (Grid reference TF568148) by selecting individual shrubs at ten metre intervals along the hedgerow length. A total of 39 hedgerow shrubs were examined. An area of bark measuring four cm^2 was removed from each shrub at 20 centimetre intervals from the bottom of the shrub upwards, and from two aspects of the shrub, that which faced the field containing *V. faba* in 2012 and from the opposite side. There were 11 standing trees within the hedgerow, all surveyed using the same technique. Bark removal continued to a maximum height of 2m and bark was replaced following examination for the presence of *B. rufimanus*. The field adjacent to the hedgerow, in which *V. faba* was grown in 2011, contained a single standing tree which was surveyed using the same technique.

A woodland edge was surveyed at Stretton in Rutland (Grid reference SK937161) in February 2013 using the bark removal technique described above for Tilney St. Lawrence. Surveys were carried out at 10 metre intervals along the woodland edge and transects were surveyed at 10 metre intervals within the wood to a distance of 20 metres from the woodland edge, providing three survey transects and a total of 135 sampling points. All hedgerows surrounding the field in which *V. faba* was grown in 2012 were surveyed, using the bark removal technique, at 10 metre intervals, providing 230 sampling points, and 43 hedgerow trees surrounding the field were surveyed using the bark removal technique to a height of 2 metres.

7.2.6 *Observations of B. rufimanus survival and emergence from seed following spring sowing*

To investigate the survival of *B. rufimanus* adults in seed, and emergence from seeds in the spring, two experiments were undertaken. On 15 March 2010 four samples of 50 *V. faba* seeds containing adult *B. rufimanus* were planted into seed and potting compost contained in plastic gravel trays. The trays were covered with transparent plastic propagation lids which were sealed with gauze to prevent emerging insects from escaping but allowing airflow into the trays. The trays were placed into an unheated glasshouse for four weeks.

A second experiment was carried out at a field site at Thornhaugh in which 100 *V. faba* seeds, replicated four times and containing adult *B. rufimanus,* were planted into a cultivated area on 16 March 2010 and covered with emergence traps to monitor adult emergence from seeds. The emergence traps were of the same design as those illustrated in Plate 7.8. These were monitored for 6 weeks.

20 seeds from the same batch as that planted were destructively tested for *B. rufimanus* viability by cutting open the seeds and removing the adults.

7.3 Results

7.3.1 *Observations of feeding behaviour prior to overwintering*

B. rufimanus adults were observed in flowering field margins surrounding the *V. faba* crop at Bourne, feeding on the pollen of other flowering plants such as thistles, mayweed, dead nettles, bindweed and ragwort following the end of *V. faba* flowering (Plates 7.10 to 7.14).

Plate 7.10: Adult *B. rufimanus* feeding in field bindweed (*Convolvulus arvensis* L.) 17 July 2009.

Plate 7.11: Adult *B. rufimanus* feeding in mayweed (*Tripleurospermum inodorum* L.) 17 July 2009.

Plate 7.12: Adult *B. rufimanus* feeding in white dead nettle (*Lamium album* L.) 17 July 2009.

Plate 7.13: Adult *B. rufimanus* feeding in creeping thistle (*Cirsium arvense* L.) 17 July 2009.

Plate 7.14: Adult *B. rufimanus* feeding in ragwort (*Senecio jacobaea* L.) 17 July 2009.

7.3.2 *Suction sampling and turf sampling*

The hedgerow from which soil and turf samples were taken was identified as non-species rich, containing *Crataegus* sp. (hawthorn), *Corylus avellana* L. (hazel), *Prunus spinosa* L. (blackthorn), *Rosa arvensis* Roth. (field rose) and *Rubus fruticosus* L. (bramble) (Appendix D). Hedgerow basal vegetation was scant in places but mean vegetation height was 30cm and leaf litter was present. All grassland habitats contained tussocky grasses such as *Dactylis glomerata* L. (cocksfoot) and *Phragmites australis* Cav. (common reed), and the grassland field corner at Crowland contained *Dipsacus fullonum* L. (teasel) as well as grass species. Mean sward height measured in the dense layer of basal vegetation was 23.9cm in the field corner habitat at Crowland, 28.3cm in the field margin at Crowland and 25.9cm in the field margin at Barnwell. From the 120 suction samples and 12 turf samples collected in January 2009 at Crowland and Barnwell from the four habitats described, no *B. rufimanus* were recorded during examination of the samples. A single adult *B. rufimanus* was recorded in the semiochemical monitoring traps at Barnwell on 05 May 2009. Eight adult *B. rufimanus* were recorded in the semiochemical monitoring traps at Crowland (Chapter 4) and the first adult was recorded on 20 April 2009.

7.3.3 *Artificial habitats – mesocosms and overwintering sleeves*

Adult *B. rufimanus* were first recorded in emergence traps covering the artificial mesocosms at Thornhaugh on 14 May 2010 (Table 7.1). Monitoring continued until 31 May but no further live adults were recorded after 17 May 2010. Adult survival in the mesocosm containing cotton wool soaked in 10% sucrose solution was lower than in the mesocosm that did not contain sucrose solution.

Table 7.1: Number of adult *B. rufimanus* recorded in emergence traps placed over the artificial winter mesocosms in spring 2010 at Thornhaugh.

Date	Number of adult *B. rufimanus* emerging from mesocosm 1	Number of adult *B. rufimanus* emerging from mesocosm 2 (containing 10% sucrose solution)
14-May-10	3	1
17-May-10	12	0
24-May-10	0	0
31-May-10	0	0

Overwintering sleeves were emptied on 20 April 2011. Live adult *B. rufimanus* were frozen for dissection. The number of surviving and dead adults was recorded (Table 7.2).

Table 7.2: Number of live and dead adult *B. rufimanus* retrieved from overwintering sleeves at Thornhaugh in 2011.

	50 adult *B. rufimanus* mixed sex unknown ratio	50 male adult *B. rufimanus*	50 female adult *B. rufimanus*	25 male and 25 female adult *B. rufimanus*
Alive	27 (15 female)	12	15	13 (7 female)
Dead	23	38	35	37

Images of the reproductive organs of *B. rufimanus* were taken from dissections of adults retrieved from the overwintering sleeves (Plates 7.15 and 7.17) and those captured in traps containing floral semiochemical attractants as they emerged from over-wintering sites at Tilney St. Lawrence in April 2011 (Plates 7.16 and 7.18). Flowering in *V. faba* occurred from the 10 May 2011 at Tilney St. Lawrence.

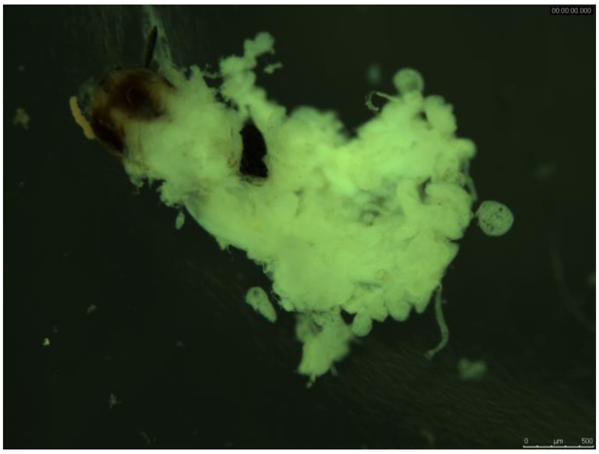


Plate 7.15: Reproductive organs of adult female *B. rufimanus* from a captive population retrieved from overwintering sleeves at Thornhaugh in 2010.

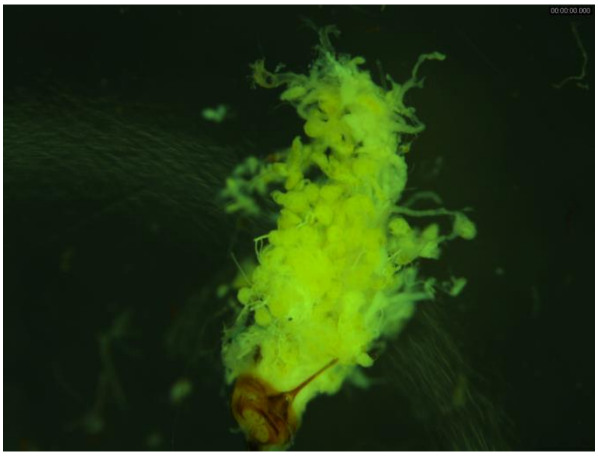


Plate 7.16: Reproductive organs of adult female *B. rufimanus* from a naturally established population captured in traps containing floral semiochemicals at emergence in 2010.

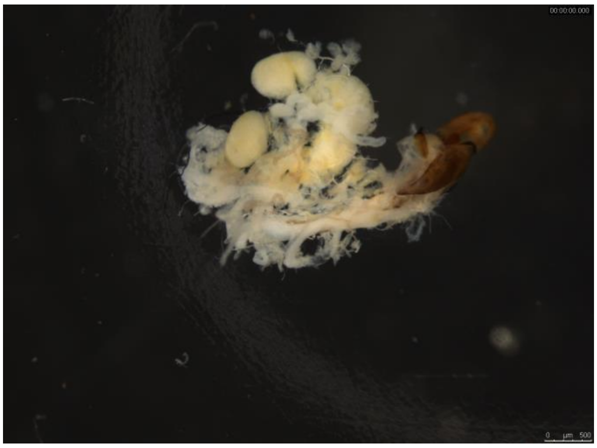


Plate 7.17: Reproductive organs of adult male *B. rufimanus* from a captive population retrieved from overwintering sleeves at Thornhaugh in 2010.

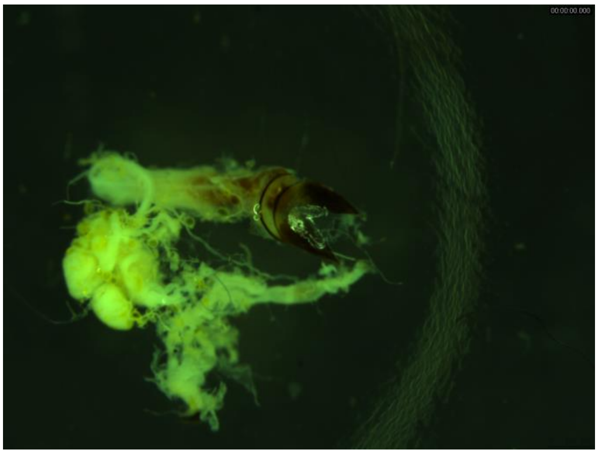


Plate 7.18: Reproductive organs of adult male *B. rufimanus* from a naturally established population captured in traps containing floral semiochemicals at emergence in 2010.

7.3.4 *Emergence traps*

No adult *B. rufimanus* were recorded in emergence traps at Crowland in 2010. Adult *B. rufimanus* were first recorded in semiochemcial monitoring traps on 11 May 2010 (Table 7.3).

Table 7.3: Number of adult *B. rufimanus* recorded in emergence traps and traps containing floral semiochemicals, at Crowland in spring 2010. Traps were located on a grass margin adjacent to a field that contained *V. faba* in 2009.

Date of inspection	Number of adult *B. rufimanus* recorded in emergence traps			Number of adult *B. rufimanus* recorded in semiochemical monitoring traps			
	1	2	3	1	2	3	4
19-Mar-10	0	0	0	0	0	0	0
24-Mar-10	0	0	0	0	0	0	0
06-Apr-10	0	0	0	0	0	0	0
09-Apr-10	0	0	0	0	0	0	0
21-Apr-10	0	0	0	0	0	0	0
30-Apr-10	0	0	0	0	0	0	0
11-May-10	0	0	0	0	1	0	0
18-May-10	0	0	0	1	1	2	0

7.3.5 *Hedgerow, woodland edge and tree surveys*

Numerous adult *B. rufimanus* were observed overwintering in trees and in dead wood at the field site at Gedney Hill in February 2011. A systematic survey of the site was not undertaken but images were recorded of adult *B. rufimanus* underneath the bark of well-established standing trees (Plates 7.19 to 7.21).

Plate 7.19: Overwintering adult *B. rufimanus* observed under the bark of a well established standing tree at Gedney Hill in February 2011.

Plate 7.20: Overwintering adult *B. rufimanus* observed under the bark of a well established standing tree at Gedney Hill in February 2011.

Plate 7.21: Overwintering adult *B. rufimanus* observed under the bark of a well established standing tree at Gedney Hill in February 2011.

The hedgerow surveyed at Tilney St. Lawrence in February 2013 measured 413 metres and contained *Crataegus* sp. (hawthorn), *P. spinosa* (blackthorn), *R. fruticosus* (bramble) and *Hedera helix* L. (common ivy) (Plate 7.22). There were 11 standing trees within the hedgerow, all were *Fraxinus excelsior* (common ash). The hedgerow was west facing on the side facing the adjacent field in which *V. faba* was grown in 2012. There was a single standing ash tree in the field. No *B. rufimanus* were recorded in hedgerow shrubs. Small numbers of *B. rufimanus* were recorded in standing trees within the hedgerow and higher numbers in the standing in-field tree (Table 7.4). All *B. rufimanus* were found on the east-facing aspects of trees.

Plate 7.22: Hedgerow at Tilney St. Lawrence surveyed in February 2012 for *B. rufimanus* presence using a bark removal technique.

Table 7.4: Total number of *B. rufimanus* recorded in hedgerow and in-field trees using a method of bark removal at Tilney St. Lawrence in February 2013.

	Number of *B. rufimanus* recorded per hedgerow tree											
Distance from base of tree (cm)	Tree 1	Tree 2	Tree 3	Tree 4	Tree 5	Tree 6	Tree 7	Tree 8	Tree 9	Tree 10	Tree 11	In-field tree
20	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	1
80	1	0	0	0	0	0	0	0	0	0	0	1
100	0	0	0	0	0	0	1	0	0	0	0	1
120	0	0	0	0	0	0	0	0	0	0	0	0
140	0	0	0	1	0	0	0	0	0	0	0	0
160	0	0	0	0	0	0	0	0	0	0	0	1
180	0	0	0	0	0	0	0	0	0	0	0	0
200	0	0	0	0	0	0	0	0	0	0	0	0

All sides of the field in which *V. faba* was grown in 2012 at Stretton were surrounded by dense species-rich hedgerows with numerous standing trees (Plate 7.23). The area of woodland surveyed in February 2013 was on the north-eastern edge of the field (Plate 7.24). All hedgerows were surveyed on both sides. There were no in-field trees.

Plate 7.23: Area at Stretton surveyed in February 2013 for the presence of *B. rufimanus* in hedgerows and woodland edge using a bark removal technique.

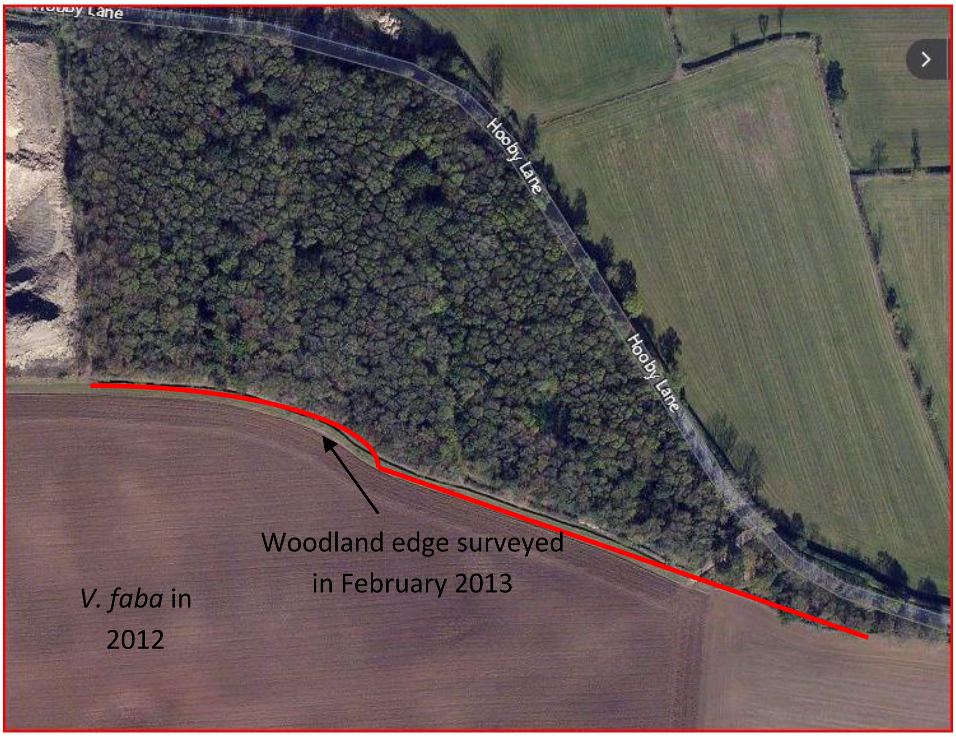


Plate 7.24: Woodland edge at Stretton in which surveys were carried out for the presence of *B. rufimanus* in February 2013 using a bark removal technique.

Few *B. rufimanus* adults were recorded in the hedgerows and trees at Stretton in 2013. All those recorded were found in the south facing woodland edge hedgerow and within the woodland on the northern edge of the field under the bark of standing, partially dead trees (Table 7.5).

Table 7.5: Number of *B. rufimanus* recorded at Stretton in February 2013 in woodland edge habitat with a description of the habitat in which they were recorded.

Number of *B. rufimanus*	Description of habitat
1	Found in a partially dead ash tree on the south facing side of the hedgerow at the field margin of the woodland. Tree radius approximately 0.3m. Found at 160cm from base of tree.
1	Found in a standing dead ash tree on the south facing side of hedgerow at the field margin of the woodland. Tree radius approximately 0.5m. Found at 140cm from base of tree.
1	Found in standing dead tree approximately 2 metres tall on the north facing side 20 m inside the woodland. Tree radius approximately 0.15m. Found at 100 cm from base of tree.
Total number recorded = 3	

7.3.6 *Observations of B. rufimanus survival and emergence from seed following spring sowing*

Of the 20 seeds containing adult *B. rufimanus* that were tested destructively to determine adult viability, all adults were found to be alive when removed from seed and following stimulation using warmth. No adult *B. rufimanus* emerged from the seeds planted into all purpose compost in gravel trays and placed into an unheated glasshouse in March 2010. No adult *B. rufimanus* emerged from the seeds planted at the field site at Thornhaugh in March 2010. This may have been due either to the adults in seed being unviable, or to conditions at the time of the experiment being unsuitable for emergence from seed. First adults were recorded in monitoring traps at Crowland on 11 May 2010 (Chapter 4, Figure 4.3), which may indicate that the experiment here, carried out from 15 March until 15 April 2010 in the glasshouse, and between 16 March and 13 May 2010 in the field was insufficient to allow emergence to occur. It is possible that although viable adults were recorded in destructive testing, that those planted into compost and soil were not viable.

7.4 Discussion

7.4.1 *Observations of feeding behaviour prior to overwintering*

B. rufimanus was found to feed in flowering plants other than host plant species following the end of the *V. faba* flowering period, indicating their ability to utilise other plant pollen resources. This may present opportunities for adult feeding earlier in the season, prior to the start of flowering in *V. faba*. Although cultural control methods for pest control include the use of weed control (Stoddard, 2010), the destruction of weeds to reduce the availability of pollen and potentially reduce the survival of *B. rufimanus,* would also reduce the availability of pollen resources for beneficial insects. The observations summarised here provide no evidence to support such an approach.

7.4.2 *Suction samples and turf samples*

Following suction and turf sampling in January 2009 of the four habitats described, there was no evidence that hedgerow base and grassland habitats were suitable for overwintering and diapause of *B. rufimanus* adults. It is possible that sampling effort was not great enough to provide sufficient material to observe the presence of *B. rufimanus* adults, although the sites were selected based on grower reports of high summer incidence of adults in *V. faba* crops and high levels of *B. rufimanus* damage to harvested *V. faba* grain from the sites. The semiochemical monitoring traps placed at Barnwell and Crowland confirmed the presence of *B. rufimanus* at both locations. The monitoring traps were not intended to verify presence of *B. rufimanus* in the grassland and hedgerow base habitats, only presence at the location. It is not possible to conclude from the 120 suction samples and 12 turf samples collected at Barnwell and Crowland for the four habitats, that grassland habitats were not suitable for overwintering *B. rufimanus.* Brook *et al.* (2008) found that suction sampling was an effective means of measuring invertebrate diversity within grassland habitats as long as sward height was taken into account. At the sites studied here the sward was tussocky and variable in height, the more dense section of vegetation varying in height between 20cm and 35cm depth. Maximum sward height was not measured, as suction and soil samples were taken only from the dense turf layer. Brook *et al.* (2008), using a Vortis suction sampler manufactured by the Burkard Manufacturing Company, found that the period required to ensure that at least 90% of beetles would be collected from a grass sward was 16 seconds. They considered the study relevant to other suction samplers as different models operate on similar principles. Suction samples were taken using high power and for a period of time that would ensure that at least 90% of beetles would be collected. Optimum sampling frequency for an individual site was between 55 and 58 sub-samples for all beetle species (classified as a single taxa and not by species) (Brook *et al.,* 2008), somewhat higher than that collected at individual sites in this study. The cumulative evidence from the four habitats sampled at Barnwell and Crowland at relatively high frequency is not sufficient to conclude that *B. rufimanus* does not overwinter in grassland habitats. The monitoring traps placed at both sites to capture *B. rufimanus* as they emerged from overwintering sites confirmed that adults were present at both locations, but possibly not in the immediate vicinity of the traps.

7.4.3 *Artificial habitats – mesocosms and overwintering sleeves*

The data from the small artificial habitat experiment were not conclusive or extensive, but they indicated the potential of *B. rufimanus* to survive within a protected habitat containing turf and leaf litter. The survival of adults within the mesocosm containing cotton wool soaked in 10% sugar solution was much lower, at 1%, than in that which did not, in which 15% survived, although the tests were not replicated. Although it was not possible to determine the reason for higher mortality since the adults were not retrieved from the mesocosms, it was possible that the presence of the sugar solution increased humidity within the protected structure for a period following the construction of the mesocosms, leading to higher mortality of *B. rufimanus*. Humidity within the mesocosms was not measured. In a natural grassland habitat, overwintering adults would be subject to high levels of climatic variation with variable levels of humidity, rainfall, light and temperature, which was not the case within the protected mesocosms. Emergence from the mesocosms occurred on approximately the same date (14 May 2010) as that on which first adults were recorded in semiochemical baited traps at overwintering sites at Crowland (11 May 2010) (Chapter 4).

Survival of adult *B. rufimanus* in the overwintering sleeves placed in hedgerow plants at Thornhaugh during the winter of 2010 to 2011 was higher than survival in the mesocosms in 2009 to 2010. Survival of adults when extracted from the sleeves was between 24% and 54% of the total population placed into the overwintering sleeves in December 2010. The proportion of males and females that survived was approximately equal. No conclusions about the suitability of hedgerow habitats as overwintering sites for *B. rufimanus* may be drawn from the limited experiment carried out, but mortality was high in the protected environment of the overwintering sleeves. Live adults extracted from the sleeves were frozen for a period and dissected to determine differences in reproductive organs compared to adults that were extracted from semiochemical monitoring traps located at Tilney St. Lawrence in April 2011 and dissected at the same time. The only differences observed following dissection were increased fatty deposits around the reproductive organs of both males and females collected at Tilney St. Lawrence, and difference in colour of the reproductive organs, those from insects collected at Tilney St. Lawrence being slightly more yellow than those from the captive population. This may indicate that the naturally established population at Tilney St. Lawrence had feeding opportunities following emergence that were not available to the captive population at Thornhaugh, and that feeding may have occurred in surrounding flowering oilseed rape crops or other non-host flowering plants. *V. faba* did not commence flowering at Tilney St. Lawrence until 10 May 2011, presenting no opportunity for naturally established *B. rufimanus* adults to feed prior to the date of collection of adults on 27 April 2011.

7.4.4 *Emergence traps*

Emergence traps placed on the grass field margin at Crowland in 2010 did not provide evidence of the presence of *B. rufimanus* within the grass habitat, although the sampling effort using emergence traps in 2010 was not sufficient to reach definite conclusions.

7.4.5 *Hedgerow, woodland edge and tree surveys*

Following the observations of *B. rufimanus* adults in trees and dead wood at Gedney Hill in February 2011, extensive surveys were undertaken at Stretton and Tilney St. Lawrence in February 2012 to determine factors that influenced the choice of overwintering sites for adults. It was found that even using extensive and laborious survey techniques, few adult *B. rufimanus* were recorded. The site at Stretton had a mixture of hedgerow and woodland habitats present, in contrast to Tilney St. Lawrence which presented rather sparse availability of potentially suitable habitat, with only a few standing trees hedgerows surrounding the field in which *V. faba* was grown in the previous year. This may explain why slightly higher numbers of adult *B. rufimanus* were recorded at Tilney St. Lawrence than at Stretton, despite substantially less sampling effort. The limited nature of the habitat at Tilney St. Lawrence may have led to higher adult density in those habitats that were present. Equally, it is possible that there were fewer adult *B. rufimanus* present at Stretton, leading to lower numbers recorded despite considerably higher sampling effort. All insects recorded were found under the bark of standing trees, and at Stretton, particularly in those containing a proportion of dead wood.

7.4.6 *Observations of B. rufimanus survival and emergence from seed following spring sowing*

The failure of *B. rufimanus* to emerge from seeds planted in March 2010, considering that all seeds contained adults, did not conclusively indicate that they do not survive the process of planting in the UK. Although it is possible that some of the adults were already dead when planting occurred, destructive sampling of seeds from the same batch as that planted did provide evidence of adult viability. According to Medjdoub *et al.* (2007), *B. rufimanus* is able to emerge from seeds that are sown in some countries, although this is dependent on temperature and soil moisture. It may be possible that soil moisture and temperature were unsuitable for survival of *B. rufimanus* during the process of planting in the UK.

Chapter 8: General Discussion and Further Recommendations

Bruchus rufimanus is one of the most important insect pests of *Vicia faba* in the UK, causing considerable damage to grain and revenue loss to growers due to the reduction of grain quality for the human consumption export market to Egypt and the Middle East and consequent loss of premium prices (Redman, 2015). Loss can be estimated using the area of *V. faba* grown and the level of damage caused per annum, and in 2014, when 30 to 40% of *V. faba* was unsuitable for export due to *B. rufimanus* damage (Frontier Agriculture Ltd., 2015, Personal Communication; Redman, 2015) and mean damage in the UK was 2.4%, loss could be estimated at between £4.03 million and £7.94 million (Chapter 2). The data in Chapter 3 showed that the level of damage varied between 2008 and 2015 and that mean percent damage for UK *V. faba* crops ranged from 1.75% to 4.98%. Damage levels were much higher than this in some regions of the UK and the factors leading to varying regional and annual levels of damage were found to be complex.

Analyses of the distribution of *B. rufimanus* using data provided by Frontier Agriculture Ltd. for damage to *V. faba* grain samples between 2008 and 2015 showed weak correlations with the indicator of host crop density, represented in this study by the number of *V. faba* samples tested per UK postcode district or county. The data contained a number of limitations which were considered when evaluating the results in Chapter 3. All samples were labelled with a postcode reference consisting of the first part of the postcode (i.e. PE8), limiting the precision with which the data could be mapped and analysed. To determine regional variations in damage, it was possible to classify samples into postcode district and county using the postcode reference, but field scale mapping was not possible. The objective of the distribution study was to determine the areas in which *B. rufimanus* populations presented the highest risk to *V. faba* crops and this was achieved, but determining a relationship between host crop density and *B. rufimanus* presence was not successful. Establishing relationships with landscape, host crop or temperature may require evaluation at a finer scale (Rusch *et al.*, 2013) or analysis using more defined scales, such as 10 x 10 kilometre or 50 x 50 kilometre squares (Quinn *et al.,* 1997). The findings showed that the range of *B. rufimanus* in the UK was not the same as the range of *V. faba*, indicating that other variables affected distribution and the presence of *V. faba* was not thought to be a constraining factor. *B. rufimanus* was shown to be endemic in many areas of England and

Wales where *V. faba* was grown, but was not present in *V. faba* samples from Scotland. There have been recent reports of incidence in Southern Ireland, which have not been captured by the data in this study as there is currently no infrastructure in Ireland to allow *V. faba* trading for the human consumption market for export, and thus samples from Ireland are not tested for *B. rufimanus* damage.

Climate is widely acknowledged as an important factor limiting the range of many insects (Samways, 1989; Quinn *et al.*, 1997; Stewart *et al.,* 2015). During the analyses of sample data for the UK, average mean daily temperature between the beginning of April and the end of July was found to explain between 3 and 13% of the variation in *B. rufimanus* damage (Table 3.2). The difficulty in correlating temperature and damage using the data available may have been caused by the unknown factors affecting the data, particularly the number of insecticide applications to each crop. It is likely that farm practice in different regions varied and growers in southern regions of the UK may have applied a greater number of insecticides due to greater risk and likely intensity of damage, although there are no data to evaluate this factor at regional level (Section 3.4). However, it is possible that the complete lack of *B. rufimanus* incidence in Scotland was due to lower average mean daily temperature during June and July compared to England and Wales.

The presence of *B. rufimanus* was studied solely using *V. faba* grain samples, potentially introducing some bias into analyses. It is possible that *B. rufimanus* was present in other *Vicia* and *Lathyrus* species in the wild or in gardens (Delobel and Delobel, 2006), but these plant species were not surveyed. The data available from the National Biodiversity Network Gateway (2016) indicated that records collected for *B. rufimanus* were from wild habitats, not arable crops. Of the 10 plant species listed by Delobel and Delobel (2006) as wild hosts for *B. rufimanus*, all but two have been recorded recently in the UK (National Biodiversity Network Gateway, 2016). The methodology used by Delobel and Delobel (2006) was to collect wild plants when pods were ripe or nearly ripe and keep the pods at room temperature for six months, monitoring bruchid emergence during that time. Huignard *et al.* (1990) showed that vitellogenesis and maturation of oocytes occurred in female *B. rufimanus* when feeding on the pollen of other *Vicia* and *Lathyrus* species. The distribution of those host plant species recorded in the UK is mainly England, Wales and southern Scotland, with only *Vicia lutea* present further north (National Biodiversity Network Gateway, 2016). This presents an added challenge when considering management strategies, as wild hosts could sustain populations in the absence of *V. faba* crops. Observations in 2009 showed that *B. rufimanus* was able to feed, following the end of flowering in *V. faba*, in other plant species (Chapter 7). Although cultural control methods for pest control include the use of weed control (Stoddard, 2010), the destruction of weeds to reduce the availability of pollen and potentially reduce the survival of *B. rufimanus,* would also reduce the availability of pollen resources for beneficial insects.

Multiple regression analyses were used to examine the relationship between temperature and *B. rufimanus* damage, and other models are available to further test the effects of temperature and other meteorological influences, such as rainfall, on *B. rufimanus* damage. Significant effects could be determined using Generalised Linear Models (GLM), Generalized Linear Mixed Models (GLMM), specifying random and fixed effects (Bates *et al*., 2015), or Generalized Additive Mixed Models that would include a temporal smoother to account for the fact that meteorological data may not be independent. Rainfall may be an important influence that was not tested in this study and should be included in further analyses. It may be appropriate to include in further study analysis of all available records of *B. rufimanus* presence including data from all UK *V. faba* traders and from wildlife records, if available, to provide a complete analysis of distribution. Further consideration of *Vicia* and *Lathyrus* plant hosts within rotations may provide information for the management of *B. rufimanus*, enabling *V. faba* growers to identify risks to crop production from the presence locally of other plant hosts.

The value of the distribution study undertaken here was to provide information to growers about the principal regions in which risk of attack from *B. rufimanus* is the highest. As part of a management strategy, this may be used to determine areas that are less suitable for production of *V. faba* for human consumption quality grain, and potentially to focus the UK *V. faba* industry on production strategies in which crops are grown for different end uses, either for animal or human consumption, in different regions, depending on *B. rufimanus* intensity.

The influence of temperature was found to be a contributing factor determining activity of *B. rufimanus* in the UK (Chapters 4 and 5). Emergence of adults from overwintering sites was suppressed when mean daily temperature during the 28 days prior to observations was below 9°C, although the interaction between temperature and photoperiod appeared to cause fluctuations in emergence that were not explained by either variable independently. Optimal conditions for adult *B. rufimanus* capture in traps occurred when mean daily temperature was greater than 15°C during the 28 days prior to observation, and photoperiod on the day of capture was greater than 17 hours (Chapter 4, Tables 4.8 and 4.9), and no adults were observed when photoperiod was below 15 hours. The effect of temperature on activity of adults within the crop, on the effectiveness of insecticide applications and on resultant damage to *V. faba* grain was mixed and it was not possible to reach firm conclusions (Chapter 5). There were indications in some years that it was possible to reduce the number of applications and maintain effective control, and that a third insecticide application did not improve control compared to a program of two well timed applications. There were indications that those plots that had two or three insecticide applications, particularly at the first pod growth stage and when a temperature threshold was reached, appeared to have higher levels of damage compared to the other treated plots, and sometimes compared to the untreated plot. This may indicate a negative effect of pyrethroid applications to beneficial insects within the crop canopy. Other studies of insect population dynamics and activity consider the interaction of variables such as plant host, resource availability, temperature, photoperiod and the presence of natural enemies when determining causes of greater or reduced activity (Delobel and Delobel, 2006; Bruce *et al.,* 2011; De Luca, 1965; Gbaye *et al.,* 2011; Huignard *et al.,* 1990; Rusch *et al.,* 2013; Stewart *et al.,* 2015; Tran and Huignard, 1992; Tran *et al.,* 1993). Oviposition may have been influenced by temperature during the two weeks immediately following the start of oviposition and the greater the temperature during this period, the greater the level of oviposition, as was seen in 2014.

When considering the effects of temperature, crop development and insecticide applications, there were practical difficulties to design trials that were suited to evaluation of management of a mobile insect species such as *B. rufimanus*. Large non-randomised plots were used in preference to a small plot randomised block design, following experience undertaking small plot screening trials to test the efficacy of insecticides for control of *B. rufimanus*. Field studies using replicated plots in a randomized complete block design often produce plot sizes too small for effective study of the target organism, as movement may occur between plots, meaning that plots are not statistically independent from the other. An experimental design to provide a more robust indication of insecticide efficacy for mobile species would be one in which each treatment plot is as large as possible and all treatments fully replicated. However, this was not practically possible, as the studies required the assistance of farmers. Some studies suggest that larger plots with reduced replication are better than small plots for assessing the effects of insecticides on mobile species (Smart *et al*. 1989) and that rapid reinvasion of small plots following treatment means that only short-term effects can be monitored (Macfadyen *et al.,* 2014). It was recognised that the design of the trials was insufficient to reach firm conclusions and that only trends could be observed.

When considering crop development, it was clear from this study that *V. faba* sowing date affected activity of *B. rufimanus* (Chapter 6). Crop development, particularly the time of flower and pod formation, has been shown to influence levels of damage caused by *B. rufimanus* in other studies (Medjdoub-Bensaad *et al*., 2007; Szafirowska, 2012). The results in this study showed that when *V. faba* was sown in early to mid-April in the UK, damage caused by *B. rufimanus* was significantly reduced compared to March sowings, even in the absence of insecticide applications. Sowing during the later part of March also led to reduced levels of damage compared to the beginning of March. Caution should be exercised when considering the design of the trials, although a robust design was used. Small plots of *V. faba* (10m x 2m) were sown in a randomised block design and large blocks for each sowing date were located adjacent to each other. This resulted in the presence of *V. faba* plots at different crop growth stages within the same field location, and it is possible that *B. rufimanus* may have shown preference for those that formed pods first, possibly introducing an element of bias into the results so that oviposition was greatest on those plots in which pods formed earlier. In a commercial context it is unlikely that *V. faba* crops would be present at different crop growth stages on a single farm, or within a field. However, the trial data were highly significant and provided sound evidence of the influence of sowing date on resultant damage to crops. These findings have been incorporated into advice to growers, using the Optibean tool (PGRO, 2015), as an option to improve management of *B. rufimanus* and potentially reduce the number of insecticide applications made to *V. faba.* This should be balanced against the yield reduction that may occur when *V. faba* is sown during April (Chapter 6) (PGRO, 2015) and growers in the UK should consider measures to improve yields when sowing during April to allow improved management of *B. rufimanus* using techniques other than insecticide applications. Some growers report adequate yield in *V. faba* sown in April when using reduced tillage or no tillage techniques to conserve moisture, and it is known that soil moisture is one of the most important factors affecting yield in *V. faba* (Sprent *et al.*, 1977; De Giorgio and Fornaro, 2004; Jensen *et al.,* 2010). Further investigation should be undertaken to provide information about the influence of tillage techniques on *V. faba* yields and soil moisture content.

It may also be advisable to incorporate the use of different sowing dates into cropping regimes for *V. faba* at a field scale, allowing growers to use a trap-cropping technique to improve management of *B. rufimanus*. Trap cropping is a traditional technique used to manipulate agricultural ecosystems, providing differential conditions for oviposition and feeding, and diverting and intercepting target species in order to reduce impact in the main crop (Shelton and Badenes-Perez, 2006). For *B. rufimanus* the use of 'perimeter trap cropping' may provide a useful solution to help reduce damage to crops, where *V. faba* is sown around the field margins at an earlier date than the remaining crop to attract *B. rufimanus* adults as they emerge from overwintering sites and to provide an earlier location for pollen feeding and oviposition. Differential insecticide programs within the field may include reduced applications to the main crop with the current standard recommended applications at the field margins, reducing overall insecticide use. In regions where incidence of *B. rufimanus* is lower, such as northern areas of England, it may be possible to eliminate insecticide applications to main crops, treating only the field margins. The effectiveness of trap cropping for the improved management of *B. rufimanus* should be further investigated.

During this study *V. faba* cultivar affected the level of damage caused by *B. rufimanus* and the commercial cultivar Fuego showed higher levels of damage than the cultivar Fury. Further study is required to determine the mechanisms that led to these differences, which may be associated with plant architecture, flowering period and abundance and the timing of pod formation (Ebedah *et al.*, 2006; Szafirowska*,* 2012). Pod characteristics should be further studied following more recent evidence of pod wall and seed testa resistance in *P. Sativum* to *B. pisorum* (Aryamanesh *et al.*, 2014).

Plant density affected the level of damage caused by *B. rufimanus* and as plant density increased so did the levels of damage. It may be expected that increased flower density in higher density plant stands would be more attractive to *B. rufimanus,* resulting in greater damage, but also that damage may be influenced by the reduction in pod availability at low plant density, leading to greater oviposition per pod and greater damage.

Studies of overwintering behaviour of *B. rufimanus* were inconclusive, and no *B. rufimanus* adults were observed in turf and soil samples. It is not possible to conclude however that adults did not overwinter in grassland habitats, such as grass and flowering field margins established for the benefit of ground-nesting birds and other invertebrates, or in basal hedgerow vegetation. Greater sampling effort would be required to provide conclusive evidence for over-winter suitability of grass margins. *B. rufimanus* adults were observed in standing mature trees, particularly those containing an element of dead wood. In some regions it may be possible to locate *V. faba* crops in areas where trees are sparse or absent to help to reduce migration from overwintering sites, but in the UK this is unlikely to be a helpful strategy due to the abundance of trees and wooded areas, and the suggestion that *B. rufimanus* is able to fly considerable distances to reach *V. faba* crops (Hoffman *et al.,* 1962).

In summary, further research is required, and recommendations for an improved strategy for the management of *B. rufimanus* in UK *V. faba* crops, as well as further investigations that may be developed from this study, are given as follows:

- Consider growing *V. faba* for the export market for human consumption in those regions which have low populations of *B. rufimanus*, such as northern England, or where *B. rufimanus* is currently not present, in Scotland.
- Consider the presence of other plant hosts (genus *Vicia* and *Lathyrus*) within rotations or in local habitats to identify increased risk to crop production. Avoid planting *V. faba* in areas close to other host plant species.
- Further work is being undertaken in the UK to develop the use of semiochemical attractants for monitoring and mass capture of adult *B. rufimanus.*
- Consider further study of parasites of *B. rufimanus* as a means to control damage. There is no current research that identifies any egg parasites.
- Undertake glasshouse or laboratory studies to further investigate the effect of temperature on oviposition.
- Undertake a comprehensive study to investigate the effects of insecticide applications in *V. faba* on beneficial insects.
- Sowing *V. faba* during late March and early April may lead to reduced damage caused by *B. rufimanus*. Yields may be reduced, and further study of options to improve yields in late sown crops, such as reduced cultivations, should be considered.

- The use of earlier sown *V. faba* as ‘perimeter’ trap crops adjacent to field margins, and later sown *V. faba* as the main crop, should be investigated for potential to reduce insecticide applications to the main crop by attracting adult *B. rufimanus* to the perimeter areas. In areas that have lower *B. rufimanus* populations, such as northern England, this may be particularly useful.

Appendices

Appendix A

Key Crop Growth stages for *V. faba* (Knott, 1990)

Code	**Description**
Germination and emergence	
000	Dry seed
001	Imbibed seed
002	Radicle apparent
003	Plumule and radicle apparent
004	Emergence
005	First leaf unfolding
006	First leaf unfolded
Vegetative stage	
101	First node
102	Second node
103...	Third node...
1n	n, last recorded node
Reproductive stage	
201(1)	Flower buds visible
203(1)	First open flowers
204(1)	First pod set
205(1)	Green pods fully formed
207(1)	Pod fill, pods green
209(1)	Seed rubbery, pods still pliable, turning black
210(1)	Seed dry and hard, pods dry and black
Pod senescence and seed ripening stage	
301	10% pods dry and black
305...	50% pods dry and black...
308	80% pods dry and black, some upper pods green
309	90% pods dry and black, most seed dry
310	All pods dry and black, seed hard
Stem senescence	
401...	10% stem brown/ black, or most stem green
405	50% stem brown/ black, or 50% stem green
410	All stems brown/ black, all pods dry and black, seed hard

Appendix B

Table I: Mean *B. rufimanus* damage recorded as percentage number of damaged *V. faba* grains per sample, for each postcode area in 2008. Means were calculated using original data provided by Frontier Agriculture Ltd. (2015).

Postcode area	Mean % damage	Postcode area	Mean % damage
AL3	3.333333333	DL6	1
B75	0.333333333	DN14	1
B78	2	DN20	5
B79	1.5	DN21	1.5
B96	1.666666667	DN3	1
BA11	2	DN36	1
BA12	0	DN9	1.5
BA22	0.666666667	DT10	2
BA8	4	DT11	1.285714286
BN17	1	DT2	0.285714286
CB10	3	DY14	0
CB25	1	EX17	0
CB5	5.5	GL11	0.5
CB7	1	GL18	0
CB8	1	GL20	1
CB9	2.384615385	GL54	0
CM1	7.8	GL6	0
CM13	9	GL7	0.5
CM22	3.571428571	GL8	0.666666667
CM3	1.0375	GU31	0.181818182
CM4	5	GU34	0.666666667
CM5	3.5	HP18	1.5
CM6	3.8	HP4	3
CM7	2.333333333	HR1	0
CO10	2.777777778	HR2	0
CO3	5.5	HR4	0
CO5	3.75	HR6	0
CO6	2.5	HR7	0
CO9	3	HR9	0
CT15	0	HU11	2
CT18	0	HU15	7
CT3	0	IP13	0.4
CV13	1	IP14	2.166666667
CV23	0.5	IP17	0
CV32	1	IP19	2
CV34	2	IP21	1.25
CV47	3	IP25	0
CV7	2	IP29	2.166666667
CW9	2	IP30	0.333333333
DE13	0.5	IP31	2.166666667

DE73	1.333333333
DE74	1
LE14	1.5
LE15	1.4
LE16	2
LE17	3
LE7	0.5
LN11	1
LN2	2
LN5	2.666666667
LN6	1
LN7	1
LN8	3
LS24	1
LU2	2.5
LU5	1
LU7	1.857142857
ME13	1
ME9	0
MK17	7
MK19	1.666666667
MK43	5.333333333
MK44	1.714285714
MK46	1
N1	0
NE36	2
NG11	1
NG12	1
NG13	2
NG24	1
NG33	2.5
NG34	1
NN11	1
NN14	1.333333333
NN15	1.2
NN17	1
NN29	5.333333333
NN3	2
NN6	1.266666667
NN7	4
NN9	3
NP18	2
NP26	0
NP7	2
NR11	1.333333333
NR14	3
NR15	5
NR16	2
IP7	1.25
IP8	5
NR19	2
NR20	34.2
NR24	0
NR25	0.5
NR28	0
NR33	0
NR34	0.375
NR35	1
NR9	0.8
OX11	0
OX12	1.25
OX13	0.714285714
OX15	8
OX17	1
OX2	5
OX25	1.125
OX29	1.5
OX44	1.333333333
PE10	1
PE15	4
PE19	1
PE26	7.5
PE28	1.6
PE8	2
PE9	2
PO17	0
PO7	4
RG17	0.666666667
RG20	0.352941176
RG24	0
RG25	2
RG26	0
RG29	2.222222222
RG4	1.333333333
RG8	2
S26	1.5
S72	3
SA62	0
SG11	17
SG18	6
SG19	3
SG2	5
SG5	1.75
SG9	3
SN10	0
SN11	2

NR17	0.5	SN14	1
NR18	3	SN16	0
SN4	1	TN12	1.5
SN6	0.666666667	TN14	4
SN7	1	TN17	5
SN8	0.2	TN25	0
SO20	0	TN29	1
SO21	1	TN30	8.125
SO24	0	WF8	2
SP11	2.2	WR12	0
SP4	0.666666667	WR13	2
SP5	1.75	WR14	1.2
SP7	0	WR2	0
SS17	12	WR5	1
SS3	2	WR6	0
SS6	2.5	WR7	0.5
SY4	0	WS13	1
TD15	0	WV16	0
TN11	1	YO19	1

Table II: Mean *B. rufimanus* damage recorded as percentage number of damaged *V. faba* grains per sample, for each postcode area in 2010. Means were calculated using original data provided by Frontier Agriculture Ltd. (2015).

Postcode area	Mean % damage	Postcode area	Mean % damage
AL3	3	CM2	3
B46	2	CM22	17
B50	10	CM3	8
B78	1	CM4	13
B79	3	CM5	12.36364
B96	8	CM6	9
BA11	6	CM7	1.666667
BA12	1.222222	CM77	9.666667
BA22	12.66667	CM8	6.666667
BA9	3	CM9	15
BH21	2.5	CO10	3.888889
BS35	2.666667	CO3	7
CB10	7.444444	CO5	9.75
CB11	2	CO6	4.6
CB2	5	CO7	4
CB21	2.333333	CO9	6.533333
CB22	11	CT15	4
CB23	4	CT3	8
CB25	9.333333	CV12	2
CB7	1.5	CV23	2.2
CB8	4	CV33	3.25

CB9	5.863636	CV34	3
CM1	10.16667	CV35	1.333333
CM12	3.5	CV47	5
CM13	16.4	CW6	1
DE65	2.5	IP19	2.5
DE73	12	IP20	1.692308
DL8	1	IP21	2.428571
DN14	1.25	IP22	6.857143
DN15	3	IP23	2.8
DN20	1.5	IP25	6
DN21	15	IP26	5
DN36	1	IP29	2.714286
DN39	10	IP30	1.125
DN40	7	IP31	7.5
DN41	4	IP5	7
DN5	3	IP6	2
DN6	4	IP7	3.095238
DN8	3	IP8	4
DN9	2	KT24	10
DT11	4.153846	L39	3.333333
DT2	1.375	LE12	3
DT9	5.333333	LE14	2.75
DY12	1	LE15	3.8
DY14	0.333333	LE16	3.25
DY9	3	LE17	1
EN5	5	LE2	2
GL18	2.75	LE65	1.666667
GL19	2.666667	LE7	1.2
GL20	1.894737	LE8	2
GL51	2	LN1	4.75
GL55	1.25	LN11	1.285714
GL6	0.5	LN12	1.5
GL7	2.333333	LN3	3
GL8	0.666667	LN4	3
GU10	13.5	LN5	10.33333
GU34	4.294118	LN6	5.333333
GU35	6	LN7	4.25
HP16	3.5	LN8	4.833333
HP17	4	LN9	2
HP18	2	LS24	1
HP5	4	LU6	3.75
HP7	9	LU7	2.714286
HR2	0.909091	ME13	8.666667
HR8	0.2	ME16	11
HU11	2.5	MK17	6.5
HU12	2	MK18	2
HU19	1	MK19	3.125
IP11	7	MK43	8.666667

IP13	2.125	MK44	7
IP14	2.666667	MK45	4.75
IP17	0.666667	NG13	5
IP18	1	NG22	8
NG24	4	PE19	4
NG32	4.25	PE21	0.666667
NG33	4	PE22	1.5
NG34	0.666667	PE23	2.333333
NN10	2	PE24	3
NN11	6	PE26	7
NN12	3.166667	PE28	7.5
NN14	7.5	PE33	7
NN17	4	PE34	2.8
NN3	6	PE37	1.5
NN6	2.142857	PE6	8.333333
NN7	1.75	PE8	5.666667
NR10	1.142857	PE9	6
NR11	0.333333	PO30	11
NR14	0.666667	PO31	4.666667
NR15	1.25	PO38	16
NR16	2.5	PO7	1.555556
NR17	5	RG14	1
NR18	3.666667	RG17	3.2
NR19	0.666667	RG18	4
NR20	2.714286	RG20	2.222222
NR21	0.333333	RG25	4
NR25	0.5	RG29	8
NR28	4	RG4	9.2
NR33	1	RH14	8
NR34	1.5	S12	3
NR35	1.833333	S26	12
NR9	2.2	S63	3
OX10	1.5	S71	3.5
OX12	4.0625	SA62	1
OX13	2	SG18	4
OX15	1	SG19	11.2
OX17	0.833333	SG9	3.25
OX20	1	SN10	8.666667
OX25	2.931034	SN14	8
OX27	5	SN16	2.909091
OX29	5	SN5	4
OX3	2.666667	SN7	1.263158
OX33	6.333333	SN8	1.818182
OX44	8.25	SO20	8
OX49	2.6	SO21	2.454545
OX7	0.666667	SO23	4
OX9	5.5	SO24	4
PE10	7.75	SO32	4.666667

PE11	10	SP11	5
PE12	6.181818	SP4	2
PE13	4	SP5	2
PE14	6	SS17	9
SS3	21	WR13	3.076923
SS5	19.66667	WR14	10.66667
TA15	2	WR2	4
TN12	21	WR6	1.25
TN14	5	WR7	6
TN25	9	YO13	1
TN27	12	YO19	1.5
TN30	2	YO25	1.5
TN33	6	YO41	1.666667
TN7	12	YO42	1
TQ14	1	YO51	1
WA14	1	YO61	1
WF3	1	YO62	1
WR12	4.5	YO8	3

Table III: Mean *B. rufimanus* damage recorded as percentage number of damaged *V. faba* grains per sample, for each postcode area in 2011. Means were calculated using original data provided by Frontier Agriculture Ltd. (2015).

Postcode area	Mean % damage	Postcode area	Mean % damage
AL3	7	CM6	7.666667
B75	5	CM7	3.75
B78	4	CM77	9.4
B96	15.2	CM8	11.5
BA12	1.333333	CM9	9
BA2	2	CO10	4.416667
BA22	10	CO5	11
BA9	1	CO6	2
BH21	0.5	CO7	6.333333
BS35	1	CO9	3
CB10	10.33333	CT14	2
CB11	6	CT3	3
CB21	3.166667	CV12	1.333333
CB22	14	CV13	5.75
CB23	3.5	CV21	1
CB25	17.83333	CV23	6.222222
CB7	4	CV32	5.333333
CB8	2.5	CV36	1
CB9	2.2	CV37	6.4
CH4	0.5	CV47	3.5
CM1	8.5	CV7	5.5
CM11	3	CV9	3

CM12	8.625	DE12	7
CM13	18.5	DE6	1
CM3	9.714286	DE72	14
CM4	4.666667	DE73	15.5
CM5	17	DL6	1.181818
DN14	4.2	IP21	10.5
DN15	18	IP22	5
DN17	1	IP23	8.5
DN20	10	IP25	6.333333
DN21	4.333333	IP26	12
DN22	5	IP29	8
DN9	30.25	IP30	4.75
DT10	9	IP31	6.875
DT11	2.4	IP6	3.166667
DT2	4.5	IP7	5
DT3	1	IP8	10
DY14	2.333333	IP9	3
EN2	11.33333	L39	3.666667
EN5	4	LE12	7
GL18	5.5	LE14	5.909091
GL19	5.5	LE15	3.454545
GL20	15	LE16	4.333333
GL51	5	LE17	9
GL56	8.666667	LE2	1.5
GL6	1	LE3	7
GL7	4.6	LE67	4
GL8	4.142857	LE7	3.384615
GU10	2	LE8	5
GU31	1.111111	LN1	9.75
GU32	5	LN10	13
GU34	1	LN11	1.857143
GU35	3	LN12	1.8
HP16	2	LN3	3
HP17	4	LN4	2.25
HP18	2	LN6	12.25
HP22	12.5	LN7	9
HP4	4.571429	LN8	4.066667
HP5	4.5	LN9	6.25
HR1	2.25	LS22	5.5
HR2	0.9	LS26	6
HR5	1	LU6	15.66667
HR8	1.666667	LU7	3.117647
HR9	1.5	ME16	9
HU11	3.1	MK17	3.428571
HU12	0.666667	MK18	2.666667
HU17	9	MK19	3.571429
HU7	10	MK43	11.83333
IP10	1.5	MK44	10.09091

IP13	5.24	NE27	0.666667
IP14	2.5	NG11	2.6
IP17	3.2	NG12	4
IP19	3.8	NG13	8
IP20	6.5	NG22	8.8
NG23	2	PE19	6.428571
NG24	6	PE21	2.142857
NG25	5.666667	PE22	5
NG32	3.4	PE24	0.5
NG33	8.5625	PE26	10
NG34	12.72727	PE28	9.666667
NN12	3	PE32	7
NN14	4.125	PE34	4
NN17	4	PE6	12.61538
NN18	4	PE7	32
NN29	13	PE8	7
NN6	2.9	PO10	18
NN7	4.571429	PO18	1
NN9	9	PO31	6
NP15	0.5	PO8	1
NP16	1	RG17	5.75
NR10	2	RG20	3.833333
NR11	1.4	RG24	1
NR14	3	RG25	5
NR15	4.2	RG26	5.5
NR16	7	RG8	1
NR18	7	S12	1
NR20	0.75	S26	11
NR21	2	S81	2
NR25	3	SG18	11
NR28	1.333333	SG19	16.66667
NR35	3.857143	SG8	6
NR9	4.4	SG9	2.833333
OX10	0.5	SL4	3
OX12	3.8	SN10	8
OX13	2.25	SN11	6
OX14	1.473684	SN14	10
OX15	2.5	SN16	6.5
OX17	1.642857	SN4	7
OX18	3.857143	SN5	11
OX25	3.30303	SN6	0.666667
OX27	0.666667	SN7	4.526316
OX29	6.716667	SN8	2.4
OX33	1	SO21	6.5
OX44	7.75	SO23	3.333333
OX49	8.5	SO24	1.2
OX5	14.66667	SO32	5.333333
OX7	2.4	SP1	4

OX9	6	SP11	4
PE10	8	SP5	4
PE11	14	SS11	8
PE12	10.66667	SW4	4.333333
PE14	5.6	SY14	1
TA4	4	WR6	5.2
TD12	5.5	WR7	15
TD15	0.333333	WR9	5
TN11	14	WS13	1
TN26	5.333333	YO12	3
TN30	6	YO16	1
TN32	10	YO17	1.2
TN7	2	YO19	2
WA14	5	YO23	0.6
WA16	0.833333	YO25	0.4
WA5	1.5	YO41	3.5
WA8	5	YO43	6
WR13	6.5	YO62	0.25
WR15	0.666667	YO8	2
WR2	7.5		

Table IV: Mean *B. rufimanus* damage recorded as percentage number of damaged grains per *V. faba* sample, for each postcode area 2012. Means were calculated using original data provided by Frontier Agriculture Ltd. (2015).

Postcode area	Mean % damage	Postcode area	Mean % damage
B50	2.5	CT18	2
BA12	1	CT5	4
BA22	9	CV13	6
CB10	9.333333	CV21	6
CB2	6.5	CV23	0.75
CB23	9.5	CV34	5.5
CB3	10	CV35	10
CB7	13.5	CV47	3.5
CB8	1.25	DE65	23
CB9	1.666667	DE74	3
CM1	14.66667	DH7	0.333333
CM11	4	DN11	1
CM12	15.5	DN20	3.5
CM13	5	DN22	1
CM3	8.333333	DN36	3
CM4	5	DN7	3.75
CM5	7.818182	DN9	19
CM6	10	DT11	3.666667
CM7	4	DT2	2
CM77	6	DT3	8

CM8	16
CM9	7
CO10	0.75
CO5	7
CO7	7.857143
CT14	4.4
GL54	1.5
GL7	3
GL8	14
GL9	6
GU28	4
GU32	3
GU34	1.555556
GU35	2
GU7	2
HP17	7.5
HP18	7.75
HP22	25
HP4	8
HP5	1
HP6	5.333333
HR1	4.8
HR2	0.25
HR9	3
HU17	0.5
IP10	5
IP13	5.5
IP14	6.5
IP19	2
IP20	1.666667
IP21	5.363636
IP22	12
IP23	2.333333
IP25	10
IP29	4
IP30	1.75
IP31	7.333333
IP6	9.333333
IP7	3
L39	1.5
LE12	6
LE14	4.4
LE15	5
LE7	1
LE8	4.666667
LE9	4
LN1	7
LN11	1.833333
DY14	5
GL11	2
GL18	6
GL19	6.666667
GL20	4.666667
GL51	6.5
LN9	1.166667
LS26	1
LU6	0.5
LU7	0.833333
ME2	18
ME6	8
ME9	5.28
MK17	4.5
MK18	1
MK19	0.75
MK43	9
MK45	11.75
NG1	1
NG10	6
NG11	6
NG12	6.2
NG13	3.714286
NG21	1.5
NG22	0.166667
NG23	10
NG32	2.5
NG34	4.333333
NN12	7
NN14	5
NN6	3.75
NN7	6.714286
NN9	1.5
NR11	2
NR14	8
NR16	2
NR17	3
NR18	4.5
NR19	0.5
NR20	26
NR34	4
NR35	1
NR9	7.176471
OX10	3.666667
OX12	2
OX13	3.6
OX14	4
OX17	2.454545

LN12	1	OX2	3
LN3	2.333333	OX25	4
LN4	5.2	OX29	2
LN6	1	OX44	4
LN7	5.333333	OX49	5
LN8	1.875	PE10	7.333333
PE11	9	SO32	3
PE12	6	SP1	1.333333
PE13	15.5	SP10	2
PE15	21	SP11	2
PE19	5.2	SP5	2.666667
PE22	3	SW1X	0.666667
PE32	3	TA11	4
PE6	10.33333	TA24	1
PE7	4	TD12	6
PO18	3.5	TN11	4
RG17	0.875	TN25	2.333333
RG20	5.066667	TN26	0.4
RG25	1	TN30	2.666667
S44	1	TN7	2
S71	8	WA13	2.5
S72	10	WA14	1
SG14	16.5	WF3	1
SG18	7	WR10	1
SG19	11	WR13	6.111111
SG3	2.2	WR2	4
SL4	16	WR6	1
SN10	10	WS13	2.6
SN11	16	YO17	0.333333
SN16	2.4	YO19	3
SN5	4	YO25	0.285714
SN7	4.923077	YO26	1.25
SN8	3.076923	YO41	0.5
SO21	3.6	YO43	2
SO23	5.75	YO62	0.4
SO24	6.666667	YO7	0.5

Table V: Mean *B. rufimanus* damage recorded as percentage number of damaged grains per *V. faba* sample, for each postcode area 2015. Means were calculated using original data provided by Frontier Agriculture Ltd. (2015).

Postcode area	Mean % damage	Postcode area	Mean % damage
AL3	7.111111	BH23	0.25
AL6	8	BN2	2
B50	3.111111	BN8	2.5
B61	1.833333	BS35	1.6

B78	6	CB10	7
B79	5	CB11	1
BA11	0.5	CB2	6
BA12	0.5	CB21	2
BA22	1.333333	CB24	6.25
BA9	1	CB25	4
BH21	0.166667	CB6	1.428571
CB7	3.875	DN10	0.75
CB8	3.666667	DN11	1.8
CB9	4.818182	DN14	1.076923
CM0	2.173913	DN17	2
CM1	5.692308	DN20	0.666667
CM11	10.25	DN21	2.625
CM12	6.5	DN22	1.333333
CM13	5	DN37	0.8125
CM16	2.5	DN6	1
CM2	2.666667	DT10	1.5
CM21	2.5	DT11	1.142857
CM23	4	DT2	2.75
CM24	1.125	DT3	1
CM3	4.166667	DT9	2
CM5	3.5	DY10	2
CM6	4	DY12	3
CM7	2.444444	EN5	3.5
CM77	2	EN9	5
CM8	1	EX32	1
CM9	0.5	GL13	10
CO10	2.466667	GL18	3.666667
CO2	2	GL19	2.375
CO4	2	GL20	4
CO5	1.6	GL54	2.4
CO7	2	GL55	3.8
CO9	2.142857	GL7	2.333333
CR6	1.25	GU10	1
CT15	4.5	GU34	0.666667
CT21	8	HA6	4
CT3	4.811111	HG1	0.333333
CT4	11.33333	HG3	1
CT5	8	HG5	8.5
CV13	1.333333	HP17	0.375
CV21	1.333333	HP18	9.75
CV23	2.692308	HP2	5.5
CV3	3	HP22	5.666667
CV33	5.333333	HP23	6
CV37	4.571429	HP4	9.5
CV47	2.8	HP5	6
CV7	2.666667	HR1	1.555556
CW6	3	HR2	1.923077

DE6	2
DE7	0.666667
DE73	2.571429
DE74	3
DL10	0.444444
DL6	0.444444
DL8	0.375
HU12	1
HU17	1.333333
HU18	1.5
IP13	3.714286
IP14	1.625
IP17	5.2
IP19	2
IP20	3.294118
IP21	5.590909
IP22	8.75
IP23	1.142857
IP25	2
IP29	2.285714
IP30	1.75
IP31	1.444444
IP6	4.666667
IP7	2.4375
IP8	4
L29	4.5
L39	3
LE12	1.333333
LE14	1.111111
LE15	2.105263
LE16	4.75
LE17	2
LE7	1.5
LE8	3
LN1	2.8
LN10	1.8
LN11	1.615385
LN12	1.529412
LN13	0.583333
LN3	4.214286
LN4	1.625
LN6	6
LN7	1.5
LN8	2.333333
LN9	1.933333
LS15	0.333333
LS22	2
LS23	4
HR4	1
HR5	0.5
HR6	1
HR7	4
HR8	2.4
HR9	2
HU11	0.285714
ME17	12.4
ME2	23.2
ME9	4.025
MK16	4.6
MK17	3
MK18	7.333333
MK19	7.166667
MK42	3
MK43	2
MK44	4.75
MK45	3.142857
MK7	3
NE66	0.5
NG10	6
NG12	2.666667
NG13	2.933333
NG19	0.333333
NG22	1.1
NG23	4.5
NG25	2.25
NG32	2.583333
NG33	2
NN11	1.666667
NN12	2.315789
NN14	1.5
NN15	1.333333
NN17	2.333333
NN29	4.142857
NN6	2.222222
NN7	3.166667
NN9	3.625
NP16	3
NR1	1
NR10	0.461538
NR11	1
NR12	3
NR14	2.166667
NR15	1.433333
NR16	2
NR17	2.25
NR18	4

LS24	1	NR20	2.857143
LS25	1.5	NR21	1
LS26	0.5	NR23	2
LU5	1	NR25	1.05
LU6	16	NR34	2.75
LU7	19	NR35	1.090909
ME13	3	NR9	1.454545
OX10	0.75	RG29	0.714286
OX11	0.2	RG4	1
OX12	1.047619	RH14	2
OX13	1	RH2	1
OX15	2	RM16	9.5
OX17	2.6	RM18	0.666667
OX18	1	S21	2
OX2	1.5	S60	4.5
OX20	17	S75	1
OX25	0.8	SG10	7
OX27	3	SG11	6
OX44	1.4	SG12	5
OX5	8.5	SG17	1
OX7	4.333333	SG18	2.666667
OX9	3	SG19	5.791667
PE10	3	SG2	5
PE11	7	SG3	3.333333
PE12	10.65714	SG4	1
PE13	11.83333	SG8	2.25
PE14	13.25926	SG9	3.875
PE15	3	SL3	1
PE19	3.6875	SL4	1
PE20	2	SN10	1.5
PE22	1.428571	SN11	0.333333
PE23	2.333333	SN14	1
PE24	1.5	SN16	1.25
PE26	5.666667	SN4	0.5
PE28	4.366667	SN6	0.666667
PE31	1	SN7	4.24
PE32	2.909091	SN8	0.75
PE33	9	SO20	1
PE34	3.333333	SO21	1
PE37	3	SO24	0.625
PE38	6	SP1	1.5
PE5	8.5	SP11	1.666667
PE6	6.933333	SP3	1
PE7	8	SP5	0.444444
PE8	2.272727	SP7	0.5
PE9	1	SS11	2
PL11	0.5	SS4	4
PO17	1.5	SS5	1.333333

PO18	0.8	SS6	3.8
PO7	1	ST21	1
PR4	0.5	SW4	3.5
RG17	0.363636	TA11	0.5
RG20	0.555556	TA15	1
RG24	1.6	TA3	1
RG25	1.888889	TD12	0.0125
TF6	2	WR6	1.933333
TN11	16	WR8	4.6
TN12	21.71429	WR9	6
TN17	3.8	WS13	2.5
TN18	9	WS14	15
TN25	15.5	WV16	1
TN26	11.5	YO13	0.333333
TN27	3.633333	YO17	1.25
TN29	4.844444	YO18	1
TS21	0.875	YO19	1
WA14	1	YO23	0.5
WA16	0.5	YO25	0.347826
WA3	5	YO26	3
WF8	0.666667	YO30	0.5
WN6	1	YO41	1
WR10	3.5	YO42	2
WR12	1.636364	YO60	1
WR13	11.72727	YO7	1.333333
WR14	9	YO8	1.75
WR2	5		

Table VI: Mean *B. rufimanus* damage recorded as percentage number of damaged grains per sample, for each UK county.

County	Mean % damage	Number of samples	Nearest Met Office Station
Bedfordshire	2.45	46	Cambridge
Berkshire	0.75862069	36	Heathrow
Buckinghamshire	1.5	18	Oxford
Cambridgeshire	2.736842105	44	Cambridge
Cheshire	2	8	Bradford
Derbyshire	1.25	6	Sheffield
Devon	0	1	Chivenor
Dorset	0.962962963	36	Hurn
East Riding of Yorkshire	2.5	30	Whitby
Essex	3.766	65	Cambridge
Gloucestershire	0.52173913	33	Ross-on-Wye
Gwent	2	2	Cardiff
Hampshire	1.085106383	58	Hurn
Herefordshire	0	43	Ross-on-Wye

Hertfordshire	3.842105263	24	Cambridge
Kent	2.806451613	33	Manston
Leicestershire	1.619047619	43	Sutton Bonnington
Lincolnshire	1.925925926	118	Waddington
Monmouthshire	0.5	5	Ross-on-Wye
Norfolk	3.961111111	50	Lowestoft
North Yorkshire	1	48	Bradford
Northamptonshire	2.378378378	57	Oxford
Northumberland	0	40	Eskdalemuir
Nottinghamshire	1.375	21	Sutton Bonnington
Oxfordshire	1.368421053	56	Oxford
Pembrokeshire	0	2	Aberporth
Shropshire	0	12	Shawbury
Somerset	1.666666667	17	Yeovilton
South Yorkshire	1.75	14	Sheffield
Staffordshire	1	15	Shawbury
Suffolk	1.683673469	114	Lowestoft
Tyne & Wear	2	2	Durham
Warwickshire	1	17	Sutton Bonnington
West Midlands	2	2	Shawbury
West Sussex	1	4	Eastbourne
West Yorks	2	2	Bradford
Wiltshire	0.442307692	71	Hurn
Worcestershire	1.086956522	35	Ross-on-Wye

Table VII: Mean *B. rufimanus* damage recorded as percentage number of damaged grains per sample, for each UK county.

County	Mean % damage	Number of samples	Nearest Met Office Station
Avon	2	4	Ross-on-Wye
Bedfordshire	6.4	44	Cambridge
Berkshire	3.6875	35	Heathrow
Berwickshire	0	25	Eskdalemuir
Buckinghamshire	3.793103448	30	Oxford
Cambridgeshire	6.333333333	60	Cambridge
Cheshire	1	6	Bradford
Derbyshire	5.666666667	7	Sheffield
Devon	1	1	Chivenor
Dorset	2.694444444	36	Hurn
Dyfed	1	1	Aberporth
East Lothian	0	8	Leuchers
East Riding of Yorkshire	1.285714286	24	Whitby
East Sussex	9	4	Eastbourne

Essex	8.867924528	107	Cambridge
Gloucestershire	1.843137255	56	Ross-on-Wye
Gwent	0	5	Cardiff
Hampshire	4.051948052	76	Hurn
Herefordshire	0.392857143	30	Ross-on-Wye
Hertfordshire	8.363636364	13	Cambridge
Isle of Wight	8.666666667	6	Hurn
Kent	8.764705882	17	Manston
Kingston Upon Hull	2	2	Whitby
Lancashire	3.333333333	3	Bradford
Leicestershire	2.15	20	Sutton Bonnington
Lincolnshire	4.540540541	174	Waddington
Norfolk	2.298245614	129	Lowestoft
North Humberside	2	8	Whitby
North Yorkshire	1.428571429	52	Bradford
Northamptonshire	3.225806452	34	Oxford
Northumberland	0	44	Eskdalemuir
Nottinghamshire	7.25	9	Sutton Bonnington
Oxfordshire	3.008403361	125	Oxford
Roxburghshire	0	7	Eskdalemuir
Rutland	3.8	6	Sutton Bonnington
Shropshire	0	8	Shawbury
Somerset	4.727272727	12	Yeovilton
South Glamorgan	0	2	Cardiff
South Humberside	3.333333333	4	Sheffield
South Yorkshire	3.909090909	23	Sheffield
Staffordshire	2	12	Shawbury
Suffolk	3.342281879	149	Lowestoft
Surrey	12.8	5	Heathrow
Warwickshire	2.666666667	21	Sutton Bonnington
West Lothian	0	4	Paisley
West Midlands	2.5	5	Shawbury
West Sussex	5.333333333	3	Eastbourne
West Yorks	0.5	6	Bradford
Wiltshire	2.320754717	54	Hurn
Worcestershire	3.783783784	37	Ross-on-Wye

Table VIII: Mean *B. rufimanus* damage recorded as percentage number of damaged grains per sample, for each UK county.

County	Mean % damage	Number of Samples	Nearest Met Office Station
Avon	1.666666667	5	Ross-on-Wye
Bedfordshire	8.941176471	59	Cambridge
Berkshire	4.612903226	34	Heathrow
Buckinghamshire	4	32	Oxford
Cambridgeshire	9.773584906	71	Cambridge
Cheshire	1.428571429	16	Bradford
Cleveland	0	5	Whitby
Derbyshire	10.6	5	Sheffield
Dorset	3	18	Hurn
Durham	0	6	Durham
Dyfed	0	1	Aberporth
East Riding of Yorkshire	2.8	19	Whitby
East Sussex	6	2	Eastbourne
Essex	8.884615385	80	Cambridge
Fife	0	10	Leuchars
Gloucestershire	5.125	27	Ross-on-Wye
Greater London	4.333333333	3	Heathrow
Gwent	0.444444444	9	Cardiff
Hampshire	3.244897959	53	Hurn
Herefordshire	1.178571429	28	Ross-on-Wye
Hertfordshire	4.277777778	20	Cambridge
Isle of Wight	6	1	Hurn
Kent	5.533333333	18	Manston
Kingston Upon Hull	4	4	Whitby
Lancashire	3.666666667	3	Bradford
Leicestershire	5.071428571	70	Sutton Bonnington
Lincolnshire	5.985185185	171	Waddington
Middlesex	11.33333333	3	Heathrow
Norfolk	4.878787879	69	Lowestoft
North Humberside	2.357142857	17	Whitby
North Yorkshire	1.245614035	91	Bradford
Northamptonshire	4.666666667	41	Oxford
Northumberland	2.4	20	Eskdalemuir
Nottinghamshire	5.25	34	Sutton Bonnington
Oxfordshire	3.52260274	153	Oxford
Powys	0	1	Shawbury
Rutland	3.166666667	6	Sutton Bonnington
Somerset	2.285714286	7	Yeovilton
South Humberside	10	1	Sheffield
South Yorkshire	17.33333333	10	Sheffield
Staffordshire	2.5	8	Shawbury
Suffolk	4.467741935	130	Lowestoft

Surrey	2	2	Heathrow
Tyne & Wear	0.666666667	8	Durham
Warwickshire	4.931034483	30	Sutton Bonnington
West Midlands	5.333333333	3	Shawbury
West Sussex	1	1	Eastbourne
West Yorks	3.5	4	Bradford
Wiltshire	4.046511628	43	Hurn
Worcestershire	6.611111111	38	Ross-on-Wye

Table IX: Mean *B. rufimanus* damage recorded as percentage number of damaged grains per sample, for each UK county.

County	Mean % damage	Number of samples	Nearest Met Office Station
Bedfordshire	4.620689655	31	Cambridge
Berkshire	3.65625	35	Heathrow
Buckinghamshire	5.684210526	29	Oxford
Cambridgeshire	9.666666667	54	Cambridge
Cheshire	1	12	Bradford
Cleveland	0	2	Whitby
Cumbria	0	1	Newton Rigg
Derbyshire	6.4	10	Sheffield
Dorset	3.2	14	Hurn
Durham	0.2	6	Durham
East Riding of Yorkshire	0.363636364	14	Whitby
East Sussex	2	2	Eastbourne
Essex	7.953125	65	Cambridge
Gloucestershire	5.115384615	28	Ross-on-Wye
Greater London	0.5	4	Heathrow
Hampshire	2.56097561	46	Hurn
Herefordshire	1.454545455	24	Ross-on-Wye
Hertfordshire	6.571428571	18	Cambridge
Kent	3.776470588	34	Manston
Kingston Upon Hull	0	2	Whitby
Lancashire	1.5	5	Bradford
Leicestershire	4.6	21	Sutton Bonnington
Lincolnshire	2.981818182	82	Waddington
Norfolk	4.380952381	80	Lowestoft
North Humberside	0	7	Whitby
North Yorkshire	0.53125	44	Bradford
Northamptonshire	5.045454545	31	Oxford
Northumberland	1.5	31	Eskdalemuir
Nottinghamshire	3.068965517	32	Sutton Bonnington
Oxfordshire	4.849450549	109	Oxford
Shropshire	0	2	Shawbury

Somerset	3.5	8	Yeovilton
South Humberside	3.5	2	Sheffield
South Yorkshire	6.545454545	14	Sheffield
Staffordshire	2.6	9	Shawbury
Suffolk	3.71875	100	Lowestoft
Surrey	2	1	Heathrow
Tyne & Wear	0	6	Durham
Warwickshire	3.681818182	24	Sutton Bonnington
West Sussex	3.6	8	Eastbourne
West Yorks	1	3	Bradford
Wiltshire	3	50	Hurn
Worcestershire	4.222222222	19	Ross-on-Wye

Table X: Mean *B. rufimanus* damage recorded as percentage number of damaged grains per sample, for each UK county.

County	Mean % damage	Number of samples	Nearest Met Office Station
Avon	1.142857143	7	Ross-on-Wye
Bedfordshire	4.095238095	63	Cambridge
Berkshire	0.487804878	41	Heathrow
Berwickshire	0	16	Eskdalemuir
Buckinghamshire	5.465517241	59	Oxford
Cambridgeshire	6.09375	163	Cambridge
Cheshire	1.538461538	13	Bradford
Cleveland	0.466666667	15	Whitby
Cornwall	0.5	2	Camborne
County Tyrone	0	4	Armagh
Cumbria	0	1	Newton Rigg
Derbyshire	2.153846154	13	Sheffield
Devon	0.333333333	3	Chivenor
Dorset	1.323529412	34	Hurn
Durham	0	22	Durham
East Lothian	0	6	Leuchars
East Riding of Yorkshire	1	27	Whitby
East Sussex	1.375	8	Eastbourne
Essex	3.299401198	170	Cambridge
Gloucestershire	3.446808511	49	Ross-on-Wye
Gwent	1.5	2	Cardiff
Hampshire	1	66	Hurn
Herefordshire	1.637931034	64	Ross-on-Wye
Hertfordshire	5.06122449	49	Cambridge
Kent	9.3328125	75	Manston
Kingston Upon Hull	1	4	Whitby
Lancashire	1.875	9	Bradford
Leicestershire	1.680851064	49	Sutton Bonnington

Lincolnshire	3.663212435	200	Waddington
Merseyside	4.5	2	Bradford
Middlesex	4	3	Heathrow
Norfolk	3.040963855	173	Lowestoft
North Humberside	0.2	20	Whitby
North Yorkshire	0.913043478	94	Bradford
Northamptonshire	2.382608696	120	Oxford
Northumberland	0.06875	33	Eskdalemuir
Nottinghamshire	2.603773585	58	Sutton Bonnington
Oxfordshire	2.318	101	Oxford
Rutland	3.5	6	Sutton Bonnington
Shropshire	1.333333333	4	Shawbury
Somerset	0.535714286	28	Yeovilton
South Humberside	0.894736842	19	Sheffield
South Yorkshire	1.666666667	18	Sheffield
Staffordshire	3.666666667	12	Shawbury
Suffolk	2.631578947	174	Lowestoft
Surrey	1.083333333	12	Heathrow
Tyne & Wear	0	3	Durham
Warwickshire	3.093023256	43	Sutton Bonnington
West Midlands	2.75	4	Shawbury
West Sussex	1.25	8	Eastbourne
West Yorks	1.153846154	13	Bradford
Wiltshire	0.779411765	71	Hurn
Worcestershire	4.459016393	65	Ross-on-Wye

Table XI: Mean annual temperature and rainfall for the years 2008, 2010, 2011, 2012 and 2015 from Met Office Regional Long-term Climate Summaries (Met Office, 2016b). Columns headed Act show the actual mean for the year. Columns headed anom show the differences from or percentage of the 30 year long-term average for 1971 to 2000.

	2008				**2010**			
Region	**Mean temp**		**Rainfall**		**Mean temp**		**Rainfall**	
	Act °C	**Anom °C**	**Act mm**	**Anom %**	**Act °C**	**Anom °C**	**Act mm**	**Anom %**
UK	9.05	0.73	1295	118	7.97	-0.62	950.5	84
England	9.84	0.77	982.1	119	8.8	-0.57	727.2	87
Wales	9.32	0.69	1663.9	119	8.15	-0.75	1127.5	79
Scotland	7.67	0.71	1720	117	6.54	-0.66	1255.4	83
N Ireland	9.06	0.61	1270.7	116	7.95	-0.73	1047.1	94
England & Wales	9.77	0.75	1076.2	119	8.71	-0.6	782.4	85
England N	9.1	0.8	1166.1	124	8.02	-0.56	827.8	87
England S	10.23	0.75	884.8	115	9.21	-0.58	674	86
Scotland N	7.36	0.68	1847.3	115	6.2	-0.7	1287.3	77
Scotland E	7.48	0.7	1314.5	118	6.36	-0.67	1107.4	97
Scotland W	8.31	0.75	1982.8	119	7.19	-0.6	1369.8	79
Eng E & NE	9.17	0.84	951.5	126	8.16	-0.44	738.3	98
Eng NW & Wales N	9.14	0.75	1549.3	121	7.92	-0.75	1027.1	79
Midlands	9.71	0.78	937.4	121	8.65	-0.58	647	82
East Anglia	10.36	0.82	684.9	114	9.39	-0.47	585.1	97
Eng SW & Wales S	9.93	0.62	1401.2	116	8.88	-0.7	995	80
Eng SE & Central S	10.46	0.75	851.1	111	9.41	-0.61	689.9	89
	2011				**2012**			
Region	**Mean temp**		**Rainfall**		**Mean temp**		**Rainfall**	
	Act °C	**Anom °C**	**Act mm**	**Anom %**	**Act °C**	**Anom °C**	**Act mm**	**Anom %**
UK	9.6	0.8	1172.5	102	8.8	-0.1	1334.8	116
England	10.6	0.9	713.1	83	9.6	-0.1	1126.1	132
Wales	9.9	0.8	1287.1	88	9.1	0	1703.3	117
Scotland	8.1	0.7	1886.4	120	7.3	-0.1	1607.8	102
N Ireland	9.4	0.5	1272.6	112	8.9	0	1186.5	104
England & Wales	10.5	0.9	792.3	84	9.5	-0.1	1205.8	129

England N	9.7	0.9	922.9	95	8.8	-0.1	1276.8	132
England S	11	0.9	602.2	76	10	-0.1	1046.5	132
Scotland N	7.8	0.7	1998.2	116	7	-0.1	1636.4	95
Scotland E	7.9	0.7	1359.4	115	7.1	-0.1	1287	109
Scotland W	8.6	0.6	2297.9	129	8.1	0.1	1913.2	107
Eng E & NE	9.8	0.9	661.7	85	8.8	-0.1	1043	134
Eng NW & Wales N	9.7	0.8	1333.7	101	8.8	-0.1	1625.2	123
Midlands	10.5	1	594	74	9.4	-0.1	1085	136
East Anglia	11.2	1	453.7	73	10.1	-0.1	810	130
Eng SW & Wales S	10.6	0.8	1068.8	85	9.8	0	1566.3	124
Eng SE & Central S	11.2	0.9	629.8	80	10.3	0	1004	127

	2015			
Region	**Mean temp**		**Rainfall**	
	Act °C	**Anom °C**	**Act mm**	**Anom %**
UK	9.2	0.4	1272.4	110
England	10.2	0.5	868.7	102
Wales	9.5	0.4	1569.4	107
Scotland	7.6	0.2	1853.5	118
N Ireland	8.8	-0.1	1323.2	116
England & Wales	10.1	0.5	965.4	103
England N	9.3	0.4	1090.2	112
England S	10.7	0.6	751.5	95
Scotland N	7.3	0.2	1983.5	115
Scotland E	7.5	0.3	1353.4	114
Scotland W	8.1	0.1	2211.3	124
Eng E & NE	9.4	0.5	815.5	105
Eng NW & Wales N	9.2	0.3	1531.8	116
Midlands	10.1	0.6	781.4	98
East Anglia	10.8	0.6	586.2	94
Eng SW & Wales S	10.3	0.5	1289.1	102
Eng SE & Central S	11	0.6	740.1	94

Appendix C

Table XII: Combined influence of sowing date, cultivar and plant density on yield at Stubton, Lincoln and Dowsby in 2015, with site as a random effect. Stubton: Sowing 1 = 06 March 2015, sowing 3 = 07 April 2015; Lincoln: Sowing 1 = 11 March 2015, sowing 3 = 10 April 2015; Dowsby: Sowing 1 = 11 March 2015, sowing 2 = 27 March 2015, sowing 3 = 10 April 2015. Cultivars are Fuego and Fury, plant densities are 20, 40, 60 and 80 plants per m^2. N = 168.

Source	Sum of Squares	Mean Square	DF	Error DF	F Value	Pr > F
Sowing date	7.4795	3.7397	2	159	9.46	0.0001
Cultivar	13.2560	13.2560	1	159	33.53	<.0001
Plant density	50.4507	16.8170	3	159	42.53	<.0001
Site (random effect)	125.6423	62.8211	2	159	158.88	<.0001
Residual	62.8670	0.3954				
Total			167			

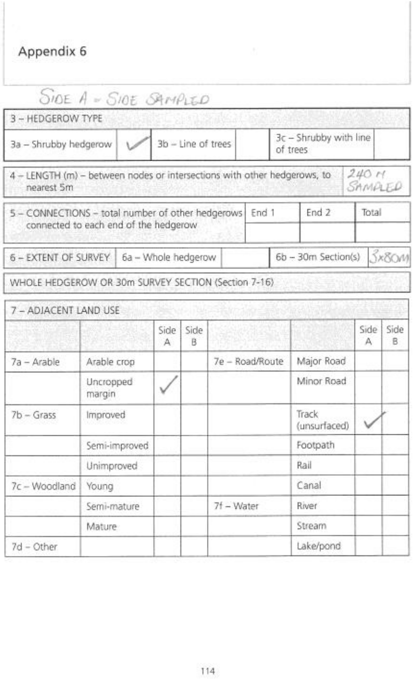
Table XIII: Pairwise mean comparison of yield at Stubton, Lincoln and Dowsby in 2015 for sowing date, cultivar and plant density, with site as a random effect. Stubton: Sowing 1 = 06 March 2015, sowing 3 = 07 April 2015; Lincoln: Sowing 1 = 11 March 2015, sowing 3 = 10 April 2015; Dowsby: Sowing 1 = 11 March 2015, sowing 2 = 27 March 2015, sowing 3 = 10 April 2015. Cultivars are Fuego and Fury, plant densities are 20, 40, 60 and 80 plants per m². N = 168.

Pairwise Mean comparison of sowing date, cultivar and plant density Difference of Least Squares Means Test adjusted using the Tukey–Kramer method				
Critical Value		0.6961		
Variable: Sowing date				
	N	Mean yield (t/ha)	Group*	Standard deviation
Sowing date 1	72	5.5385	a	1.3397
Sowing date 2	24	6.7861	a	0.7762
Sowing date 3	72	5.1051	b	1.2499
Critical value		0.4570		
Variable: Cultivar				
Fuego	84	5.2501	b	1.4114
Fury	84	5.8119	a	1.2247
Critical value		0.7920		
Variable: Plant density				
20	42	4.6358	c	1.3158
40	42	5.5925	ab	1.2986
60	42	5.7921	a	1.1528
80	42	6.1036	a	1.1897

*Means within a column with the same letter are not significantly different (p>0.05)

Appendix D

Hedgerow survey form completed for survey undertaken at Barnwell in 2009 (DEFRA, 2007).



Appendix 6

SIDE A = SIDE SAMPLED

3 – HEDGEROW TYPE					
3a – Shrubby hedgerow	✓	3b – Line of trees		3c – Shrubby with line of trees	

4 – LENGTH (m) – between nodes or intersections with other hedgerows, to nearest 5m	240 M SAMPLED

5 – CONNECTIONS – total number of other hedgerows connected to each end of the hedgerow	End 1	End 2	Total

6 – EXTENT OF SURVEY	6a – Whole hedgerow		6b – 30m Section(s)	3x80M

WHOLE HEDGEROW OR 30m SURVEY SECTION (Section 7-16)

7 – ADJACENT LAND USE

		Side A	Side B			Side A	Side B
7a – Arable	Arable crop			7e – Road/Route	Major Road		
	Uncropped margin	✓			Minor Road		
7b – Grass	Improved				Track (unsurfaced)	✓	
	Semi-improved				Footpath		
	Unimproved				Rail		
7c – Woodland	Young				Canal		
	Semi-mature			7f – Water	River		
	Mature				Stream		
7d – Other					Lake/pond		

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Field Survey Form

8 – ASSOCIATED FEATURES – See also Part B section 18

	Side A	Side B		
8a – Bank – Height (in metres) to nearest 25cm			8f – Ditch – internal – Dry (tick)	
8b – Average herb vegetation height (cm) to nearest 5cm	30cm		8g – Ditch – internal – Wet (tick)	
8c – Fence (tick)				
8d – Ditch – external – Dry (tick)				
8e – Ditch – external – Wet (tick)				

9 – UNDISTURBED GROUND (measured from the centreline of the hedgerow)	Side A	Side B
9a – Average width of undisturbed ground (m) to nearest 50cm*	150cm	
9b – Average width of perennial herbaceous vegetation (m) to nearest 50cm*	100cm	

NB * mark N/A or road etc if a road or built feature or hedge is adjacent to grassland or woodland

10 – NUTRIENT ENRICHMENT GROUND FLORA INDICATOR SPECIES

Estimate % cover of each species within a 2m wide band alongside the hedgerow (to nearest 5%)

10a – Nettles	Side A	Side B	10b – Cleavers	Side A	Side B	10c – Docks	Side A	Side B

11 – RECENTLY INTRODUCED, NON-NATIVE SPECIES

11a – RECENTLY INTRODUCED, NON-NATIVE GROUND FLORA SPECIES

	Species	Side A	Side B
% cover of introduced species in the hedge-bottom (to nearest 5% or enter just the name if <5%)			

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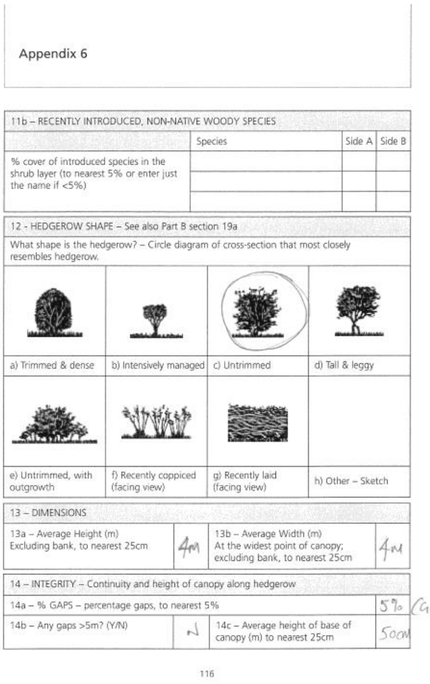

Appendix 6

11b – RECENTLY INTRODUCED, NON-NATIVE WOODY SPECIES

	Species	Side A	Side B
% cover of introduced species in the shrub layer (to nearest 5% or enter just the name if <5%)			

12 - HEDGEROW SHAPE – See also Part B section 19a

What shape is the hedgerow? – Circle diagram of cross-section that most closely resembles hedgerow.

a) Trimmed & dense	b) Intensively managed	[✓ c) Untrimmed]	d) Tall & leggy
e) Untrimmed, with outgrowth	f) Recently coppiced (facing view)	g) Recently laid (facing view)	h) Other – Sketch

13 – DIMENSIONS

13a – Average Height (m) Excluding bank, to nearest 25cm	4m	13b – Average Width (m) At the widest point of canopy; excluding bank, to nearest 25cm	4M

14 – INTEGRITY – Continuity and height of canopy along hedgerow

14a – % GAPS – percentage gaps, to nearest 5%			5% (Gateway)
14b – Any gaps >5m? (Y/N)	N	14c – Average height of base of canopy (m) to nearest 25cm	50cm

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Field Survey Form

15 – ISOLATED HEDGEROW TREES – See also Part B section 21

Use one row per specimen or one row and a number if there are many individuals of the same species in the same size class. Estimate DBH to nearest 5cm or nearest 1cm if DBH less than 5cm.

Species	DBH (cm)	Species	DBH (cm)

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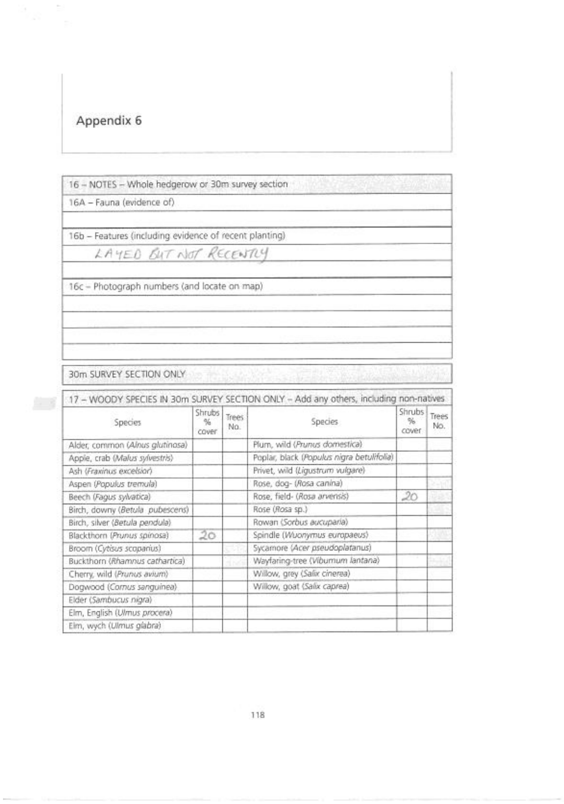

Appendix 6

16 – NOTES – Whole hedgerow or 30m survey section

16A – Fauna (evidence of)

16b – Features (including evidence of recent planting)

LAYED BUT NOT RECENTLY

16c – Photograph numbers (and locate on map)

30m SURVEY SECTION ONLY

17 – WOODY SPECIES IN 30m SURVEY SECTION ONLY – Add any others, including non-natives

Species	Shrubs % cover	Trees No.	Species	Shrubs % cover	Trees No.
Alder, common (*Alnus glutinosa*)			Plum, wild (*Prunus domestica*)		
Apple, crab (*Malus sylvestris*)			Poplar, black (*Populus nigra betulifolia*)		
Ash (*Fraxinus excelsior*)			Privet, wild (*Ligustrum vulgare*)		
Aspen (*Populus tremula*)			Rose, dog- (*Rosa canina*)		
Beech (*Fagus sylvatica*)			Rose, field- (*Rosa arvensis*)	20	
Birch, downy (*Betula pubescens*)			Rose (*Rosa* sp.)		
Birch, silver (*Betula pendula*)			Rowan (*Sorbus aucuparia*)		
Blackthorn (*Prunus spinosa*)	20		Spindle (*Wuonymus europaeus*)		
Broom (*Cytisus scoparius*)			Sycamore (*Acer pseudoplatanus*)		
Buckthorn (*Rhamnus cathartica*)			Wayfaring-tree (*Viburnum lantana*)		
Cherry, wild (*Prunus avium*)			Willow, grey (*Salix cinerea*)		
Dogwood (*Cornus sanguinea*)			Willow, goat (*Salix caprea*)		
Elder (*Sambucus nigra*)					
Elm, English (*Ulmus procera*)					
Elm, wych (*Ulmus glabra*)					

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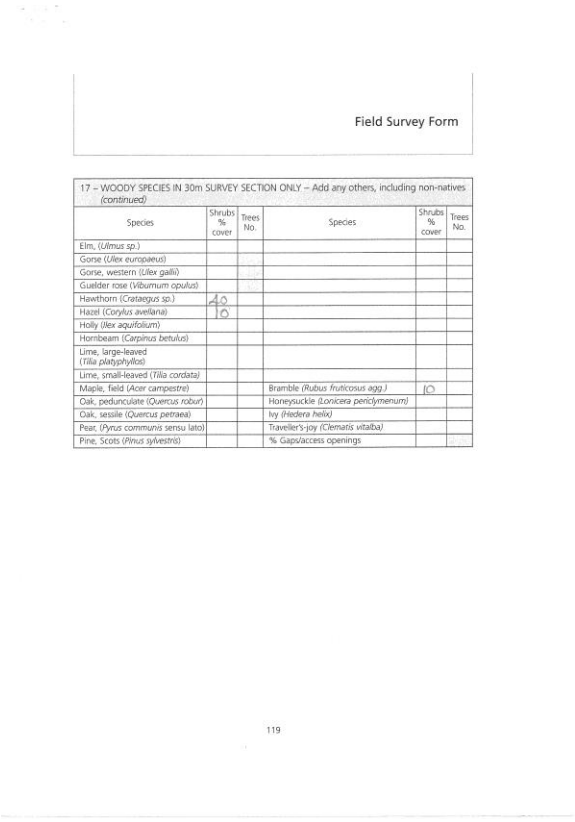

Field Survey Form

17 – WOODY SPECIES IN 30m SURVEY SECTION ONLY – Add any others, including non-natives *(continued)*

Species	Shrubs % cover	Trees No.	Species	Shrubs % cover	Trees No.
Elm, (*Ulmus sp.*)					
Gorse (*Ulex europaeus*)					
Gorse, western (*Ulex gallii*)					
Guelder rose (*Viburnum opulus*)					
Hawthorn (*Crataegus sp.*)	40				
Hazel (*Corylus avellana*)	10				
Holly (*Ilex aquifolium*)					
Hornbeam (*Carpinus betulus*)					
Lime, large-leaved (*Tilia platyphyllos*)					
Lime, small-leaved (*Tilia cordata*)					
Maple, field (*Acer campestre*)			Bramble *(Rubus fruticosus agg.)*	10	
Oak, pedunculate (*Quercus robur*)			Honeysuckle *(Lonicera periclymenum)*		
Oak, sessile (*Quercus petraea*)			Ivy *(Hedera helix)*		
Pear, (*Pyrus communis* sensu lato)			Traveller's-joy *(Clematis vitalba)*		
Pine, Scots (*Pinus sylvestris*)			% Gaps/access openings		

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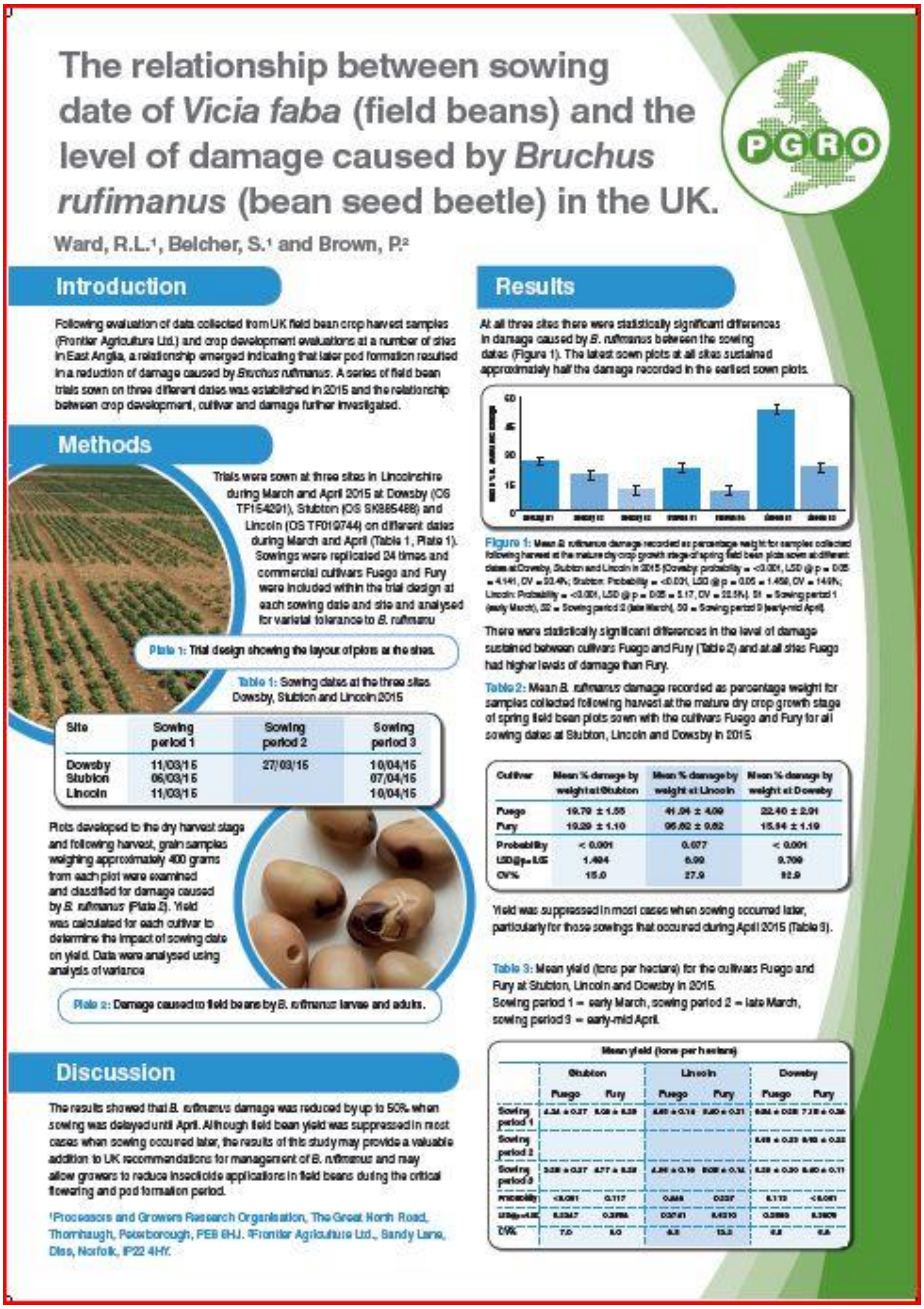
Appendix E

Recent publications



The relationship between sowing date of *Vicia faba* (field beans) and the level of damage caused by *Bruchus rufimanus* (bean seed beetle) in the UK.

Ward, R.L.[1], Belcher, S.[1] and Brown, P.[2]

Introduction

Following evaluation of data collected from UK field bean crop harvest samples (Frontier Agriculture Ltd.) and crop development evaluations at a number of sites in East Anglia, a relationship emerged indicating that later pod formation resulted in a reduction of damage caused by *Bruchus rufimanus*. A series of field bean trials sown on three different dates was established in 2015 and the relationship between crop development, cultivar and damage further investigated.

Methods

Trials were sown at three sites in Lincolnshire during March and April 2015 at Dowsby (OS TF154291), Stubton (OS SK885488) and Lincoln (OS TF019744) on different dates during March and April (Table 1, Plate 1). Sowings were replicated 24 times and commercial cultivars Fuego and Fury were included within the trial design at each sowing date and site and analysed for varietal tolerance to *B. rufimanu*

Plate 1: Trial design showing the layout of plots at the sites.

Table 1: Sowing dates at the three sites Dowsby, Stubton and Lincoln 2015

Site	Sowing period 1	Sowing period 2	Sowing period 3
Dowsby	11/03/15	27/03/15	10/04/15
Stubton	06/03/15		07/04/15
Lincoln	11/03/15		10/04/15

Plots developed to the dry harvest stage and following harvest, grain samples weighing approximately 400 grams from each plot were examined and classified for damage caused by *B. rufimanus* (Plate 2). Yield was calculated for each cultivar to determine the impact of sowing date on yield. Data were analysed using analysis of variance

Plate 2: Damage caused to field beans by *B. rufimanus* larvae and adults.

Discussion

The results showed that *B. rufimanus* damage was reduced by up to 50% when sowing was delayed until April. Although field bean yield was suppressed in most cases when sowing occurred later, the results of this study may provide a valuable addition to UK recommendations for management of *B. rufimanus* and may allow growers to reduce insecticide applications in field beans during the critical flowering and pod formation period.

[1] Processors and Growers Research Organisation, The Great North Road, Thornhaugh, Peterborough, PE8 6HJ. [2] Frontier Agriculture Ltd., Sandy Lane, Diss, Norfolk, IP22 4HY.

Results

At all three sites there were statistically significant differences in damage caused by *B. rufimanus* between the sowing dates (Figure 1). The latest sown plots at all sites sustained approximately half the damage recorded in the earliest sown plots.

Figure 1: Mean *B. rufimanus* damage recorded as percentage weight for samples collected following harvest at the mature dry crop growth stage of spring field bean plots sown at different dates at Dowsby, Stubton and Lincoln in 2015 (Dowsby: probability = <0.001, LSD @ p = 0.05 = 4.141, CV = 30.4%; Stubton: Probability = <0.001, LSD @ p = 0.05 = 1.458, CV = 14.9%; Lincoln: Probability = <0.001, LSD @ p = 0.05 = 5.17, CV = 22.5%). S1 = Sowing period 1 (early March), S2 = Sowing period 2 (late March), S3 = Sowing period 3 (early-mid April).

There were statistically significant differences in the level of damage sustained between cultivars Fuego and Fury (Table 2) and at all sites Fuego had higher levels of damage than Fury.

Table 2: Mean *B. rufimanus* damage recorded as percentage weight for samples collected following harvest at the mature dry crop growth stage of spring field bean plots sown with the cultivars Fuego and Fury for all sowing dates at Stubton, Lincoln and Dowsby in 2015.

Cultivar	Mean % damage by weight at Stubton	Mean % damage by weight at Lincoln	Mean % damage by weight at Dowsby
Fuego	19.79 ± 1.55	41.94 ± 4.09	22.40 ± 2.01
Fury	10.29 ± 1.10	36.82 ± 9.62	15.84 ± 1.19
Probability	< 0.001	0.077	< 0.001
LSD@p=0.05	1.494	6.99	3.709
CV%	15.0	27.9	32.9

Yield was suppressed in most cases when sowing occurred later, particularly for those sowings that occurred during April 2015 (Table 3).

Table 3: Mean yield (tons per hectare) for the cultivars Fuego and Fury at Stubton, Lincoln and Dowsby in 2015.
Sowing period 1 = early March, sowing period 2 = late March, sowing period 3 = early-mid April.

Mean yield (tons per hectare)

	Stubton		Lincoln		Dowsby	
	Fuego	Fury	Fuego	Fury	Fuego	Fury
Sowing period 1	4.24 ± 0.27	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Sowing period 2					[illegible]	[illegible]
Sowing period 3	[illegible]	4.77 ± [illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Probability	<0.001	0.117	[illegible]	[illegible]	[illegible]	<0.001
LSD@p=0.05	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
CV%	7.0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

PGRO AGRONOMY

UPDATE

Bruchid beetle update

The last ten years have seen bruchid damage levels in field beans fluctuate in the UK from very high levels in 2006 and 2007, to somewhat lower levels in 2013 to 2015 (Figure 1). These fluctuations are largely attributable to changes in recommendations for control of the pest, combined with differences in peak temperatures during the critical period of first pod-set and early pod-fill stages of crop growth.

BECKY WARD
PRINCIPAL TECHNICAL OFFICER

Bruchid damage

Following the difficult years of 2006-2007 PGRO worked with a number of partners in a DEFRA funded project[1], to develop a new approach to control. A prototype trap was developed to monitor field presence, spray technology was investigated to improve crop penetration of insecticides using angled nozzles, distribution of the pest was mapped, and a study was undertaken to determine whether there was any varietal tolerance to bruchids. This was followed by a series of trials co-funded by Innovate UK and industry[2] to develop a forecasting system combining the prototype trap developed in the previous project with an email alert for growers that predicts optimum spray timing (BruchidCast[*]). The recommendation for spray applications starting at first pod growth stage, when maximum daily temperatures had reached 20°C for two consecutive days was confirmed, and has helped to lower and stabilise UK levels of bruchid damage. A network of traps has been established across the UK to monitor activity of the pest, so please contact PGRO if you are interested in hosting a site in 2016.

Following this work, there were some preliminary indications that large differences in the timing of first pod-set in the period between 2011 and 2015 were leading to significant differences in overall levels of damage across the UK (Figure 2). PGRO subsequently used trials established at different planting dates[3] to study the effects of pod-set timing on levels of bruchid damage.

Three sites were established at Stubton, Lincoln and Dowsby in Lincolnshire and samples were taken at harvest and assessed for bruchid damage.

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