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SUPPRESSION OF BRUCHIDS INFESTING STORED GRAIN LEGUMES WITH THE PREDATORY BUG *XYLOCORIS FLAVIPES* (REUTER) (HEMIPTERA: ANTHOCORIDAE)

by

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Master of Science

Abstract

M. Sc. Sharlene E. Sing Entomology

Biological control of pest Bruchidae may provide an important management strategy against infestation of stored grain legumes, a key source of dietary protein in developing countries. Previous related research has focused on the potential of parasitoids to control bruchids; the role of generalist predators in this application has not yet been extensively explored.

The anthocorid true bug *Xylocoris flavipes* (Reuter) exhibited a Type II density dependent functional response to five species of adult bruchids. The rate of kill of these large prey was quite low but fairly consistent and female predators were generally more effective. Of the species examined, only the eggs and neonate larvae of *A. obtectus* were accessible and predation on these stages was high.

Population interaction studies evaluating the effects of predator density and of time elapsed between infestation of commodity and predator addition indicated that adding the predator simultaneously with the pests significantly reduced the number of F_1 bruchid progeny for all species. Predator density contributed less to bruchid suppression than time of predator addition and bruchid progeny suppression was much greater than anticipated given the rate of kill observed in the functional response experiments. Reproduction by *A. obtectus* was almost entirely inhibited by the predator.

The high levels of suppression achieved with the predator indicated a significant biological control potential; however, the more fecund bruchid species with inaccessible immature stages continued to produce a large number of progeny. The predator was then combined with larval parasitoids capable of utilizing the internally-developing stages of the bruchids; bruchid suppression was considerably enhanced over the predator alone, and for the most fecund pests, suppression was greater than for the parasitoids alone.

Résumé

M. Sc. Sharlene E. Sing Entomologie

La lutte biologique de la peste bruchidae peut pourvoir une stratégie de conduite importante contre l'infestation des légumes en grain en stockage, une source de base de la protéine dans les pays qui se développent. La recherche préalable ayant rapport a mit au point le potentiel des parasites pour lutter les bruches; le rôle des prédateurs généralists dans cette application n'est pas encore exploré d'une manière étendue.

L'anthocoride vrai punaise *Xylocoris flavipes* (Reuter) a exhibée une réponse fonctionnel avec dépendance sur densité Type II, envers cinq genres de bruches adultes. La vitesse de tue de ses proie larges était assez bas mais bien consistant et les prédateurs femelles étaient généralement plus effectif. De tous les genres examinés, seul les oeux et les larves néonates d'*A. obtectus* étaient accessible et la prédation dans ces étapes fit haut.

Les études d'interaction des populations évaluant les effets de la densité des prédateurs et du temps passé entre l'infestation de la denrée stockée et l'addition des prédateurs, ont indiqués que l'addition des prédateurs simultanément avec les pestes, a reduire d'une manière significative, le nombre des descendants de la bruche F_1 pour tous les genres. La densité des prédateurs avait moins de contribution à la suppression des bruches que le temps d'addition des prédateurs, et la suppression du descendants des bruches était plus grande qu'anticipé, donnant la vitesse de tue observée dans les expériences de la réponse fonctionnel. La réproduction d'*A. obtectus* était près que entièrement empêché par le prédateur.

Les hauts niveaux accomplis concernant la suppression avec le prédateur, ont indiqués d'une manière significative, le potentiel de la lutte biologique; cependant, les genres des bruches plus fécond avec les étapes pas mûr et inaccessible, continuent à produire un grand nombre des descendants. Le prédateur était donc combiné avec les parasites larvaires capable d'utiliser les étapes de la bruche qui se devéloppent à

l'intérieur; la suppression des bruches était considérablement enchérir que celui du prédateur seul, et pour les pestes plus fécond, la suppression était plus grande que celui des parasites seulement.

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Introduction

The dried, edible seeds of grain legumes, commonly referred to as beans or pulses, provide an abundant, inexpensively produced, resource conserving source of highly nutritional dietary protein and are essential to human survival in many developing countries (Smartt, 1990). The average protein content of grain legumes ranges between 20-26%, and a full compliment of essential amino acids is supplied when methionine- and cystine-deficient but lysine-rich legumes are consumed in conjunction with grain cereals, which are typically deficient in lysine but supply sufficient levels of methionine and cystine (Kay, 1979).

Bruchids, the seed beetles, are the primary pests of stored grain legumes (Southgate, 1978); the most destructive and economically significant species belong to the genera *Acanthoscelides*, *Callosbruchus*, and *Zabrotes* (Credland, 1994). Bruchids are entrenched at every level of the pulse ecosystem, from initial field infestation through all levels of storage and distribution (Pedersen, 1978). The majority of species used in this study, which include *Acanthoscelides obtectus* (Say), *Callosobruchus analis* (F.), *C. chinensis* (L.), *C. maculatus* (F.), and *Zabrotes subfasciatus* (Boheman), are now considered cosmopolitan in distribution (Southgate, 1978). Postharvest losses to stored grain legumes are characterized by bruchid consumption and contamination of the beans resulting in reduction of commodity weight and qualitative deterioration (Sulunkhe et al, 1985). The storage habitat facilitates rapid, catastrophic increases in bruchid populations if left unchecked, even when initial infestation is minor (Caswell, 1961).

Chemical means in the form of contact insecticides or fumigants are typically used for the prevention or suppression of bruchid infestations in stored grain legumes (Salunkhe et al, 1985). Prohibitive costs, mis-application, chemical persistence and insecticide resistance have contributed to rendering this approach inaccessible or unreliable.

Experimentation in the biological control of stored-product pests is well-documented (Arbogast, 1984; Brower et al, 1996) and expanding with the necessity of finding alternatives to chemical treatments. Three polyphagous natural enemies, the predatory bug *Xylocoris flavipes* (Hemiptera: Anthocoridae), and two larval parasitoids, *Anisopteromalus*

calandrae and *Pteromalus cerealellae* (Hymenoptera: Pteromalidae) have shown the greatest promise in this application, and are the subjects evaluated in the current research project. The primary objectives of this research were to examine the effects of the following factors on suppression of bruchid populations: predator density, time elapsed between infestation and predator addition to commodity, the separate and combined presence of the predator and parasitoid strains/species, parasitoid conditioning and natural enemy pesticide-resistance status. Determination of optimal natural enemy treatments under laboratory conditions may provide some insight into developing applied management strategies for the cosmopolitan problem of bruchid infestation in grain legumes.

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Section I: Literature Review

BRUCHIDS

Origins and distributions

Bruchids, the seed beetles, are the primary pests of stored grain legumes. They are known to breed on every continent except Antarctica (Southgate, 1979). Of the 20 bruchid species identified as pests of stored grain legumes, the most destructive and economically significant are members of the genera *Callosobruchus*, *Acanthoscelides*, and *Zabrotes* (Credland, 1994).

Callosobruchus maculatus (Fabricius), Cm, and *Callosobruchus chinensis* (Linnaeus), Cc, originated in Asia and Africa and have become established in the Americas, the West Indian and Pacific Islands, the Mediterranean region, and Australia (Southgate, 1978). The origins of *Callosobruchus analis* (Fabricius), Ca, are less clear because reported Ca specimens have routinely been found to be misidentified Cm (Southgate et al, 1957). Southgate (1978) suggests that the species probably arose in Southeast Asia and India and later became established in Africa.

Acanthoscelides obtectus (Say), Ao, and *Zabrotes subfasciatus*, Zs, are New World species thought to have originated in South and Central America. Hoffmann et al (1962) reports that Ao-infested lima beans (*Phaseolus lunatus*) were discovered among the artifacts recovered during archeological digs at the Incan necropolis in Ancon, Peru. Ao has attained a more far-reaching distribution than Zs, including the Americas, Asia, Africa, India, the Pacific and West Indian Islands, and both northern and southern European countries (Southgate, 1978). Zs has not been reported from Asia or the Australasian region, and European records are limited to Italy and Portugal (Southgate, 1978).

Classification and descriptions

The bruchids are classified as follows: Coleoptera (order); Polyphaga (suborder); and Bruchidae (family) (Borror and Delong, 1964). It is generally agreed that the Bruchidae should be placed in the superfamily Chrysomeloidea although some taxonomists place the family in the superfamily Curculionoidea (Southgate, 1979). The family Bruchidae is divided into six distinctive subfamilies, three economically significant: Amblycerinae, Bruchinae, and Pachymerinae, and three of non-pest status: Eubaptinae (Southgate, 1979), Rhaebinae (Hoffmann et al, 1962) and

Kytorhininae. Cm, Cc, Ca, and Ao are included in the subfamily Bruchinae while Zs has been classified as a member of the subfamily Amblycerinae. Complete descriptions of adult bruchids are given by: Cm, Herford (1935) and Southgate et al (1957); Cc, Southgate (1958) and Herford (1935); Ca, Southgate et al (1957) and Halstead (1963); Ao, Herford (1935), Kingsolver (1968), and Johnson (1983); and Zs, Herford (1935) and Kingsolver (1970). The common names of the species examined here are: Cm, cowpea weevil (Stoetzel, 1989) ; Cc, adzuki bean weevil; Ao, bean weevil (Stoetzel, 1989) and bean seed beetle (Singh et al, 1978); and Zs, Mexican bean weevil (Stoetzel, 1989). Ca has no common name.

Biology

Bruchids have been ethologically categorized according to the influence of habitat (field or storage) and host plant state (developing seeds or pods of growing leguminous plants, or their stored, dried seeds) on life history (univoltine or multivoltine) (Hoffmann et al, 1962). Although Cm, Cc, Ao, and Zs can initiate grain legume infestation in the ripening field crop (Labeyrie, 1981; Hagstrum, 1985; and Prevett, 1961), these species are considerably more destructive to stored, dried legumes and it is the benefits of shelter and ready food resources conferred by the storage habitat (Imura, 1990) that facilitate their multivoltine existence (Southgate, 1981). According to Pajni and Gill (1991), Ca is incapable of infesting green pods even under experimental conditions. Zs infestation occurs only in the exposed seeds of dehiscent pods because direct contact with the seeds is required to stimulate ovarian production (Pimbert and Pierre, 1983). In the wild, Cc emerging from wild legumes feed on nectar, pollen and fungi throughout the summer months until returning to the wild legumes when they begin flowering and fruiting (Yoshida et al, 1987). A comprehensive listing of pest species associated with the ripening pods and mature dry seeds of specific leguminous plants cultivated for human and/or animal consumption is provided in Kay (1979).

In the storage habitat, Cm, Cc, Ca, and Zs exhibit a common mode of oviposition and larval emergence (hatching). Larvae emerging from discoid or ellipsoid eggs cemented to the bean by the female bore through the egg shell and legume testa into the cotyledons. In contrast, ovoid Ao eggs

are deposited loosely among the infested commodity and debris, where newly-emerged larvae must successfully locate and then penetrate a suitable host seed before starving or dessicating (Howe and Currie, 1964). In most other respects, the life histories of all species discussed here are fairly similar: larvae continue to feed and tunnel, usually molting four times before pupation. Late instar larvae concentrate feeding on a small region directly below the testa forming a visible, distinctive 'window' which the emerging adults will push through with their legs and head to escape the host seed. Adults are relatively short-lived and begin to reproduce soon after emergence. The adults are not known to feed to any appreciable degree on stored legumes, but egg production increases when water or nectar is available (Szentesi, 1972). The effects of various environmental conditions on the life history of stored product Bruchidae are reported in Howe and Currie (1964). All stages of the species discussed here are susceptible to extremes of temperature (below 17.5° and above 32.5° C) and humidity (below 50% and above 80% relative humidity). Hoffman et al (1962) provide an exhaustive guide to the developmental biology of all bruchid species of agricultural significance.

Polymorhphism

Utida (1974; 1981) discussed the occurence of phenotypic and behavioral polymorphism in Cm where both atypical, 'active', flight or distributive forms with reduced reproductivity, and 'normal' or non-flight, highly prolific forms arise in the same population due to combined environmental and genetic influences. Taylor (1974) contends that flight form females are produced in increasingly higher numbers with time accrued in storage of cowpeas; the build-up of insect populations, temperature and reduction of resources demanding some form of dispersal mechanism. Monge et al (1991) correlates flight and non-flight forms of Cm to climatic cycle: the flight form appears during the rainy season, locating and colonizing cowpeas in the field at a relatively low rate of infestation, while the non-flight form is highly prolific in stored cowpeas during the dry season.

Economic significance

Role of grain legumes in human nutrition

Grain legumes, also generically known as pulses or dried beans and

peas, are often the only source of affordable and accessible dietary protein for the human population inhabiting temperate and semi-tropical developing countries (Smartt, 1990). The nutritional composition of food legumes includes 20-30% protein, 1-7% lipids, 24-68% carbohydrates, and additionally provides a good source of minerals including calcium, iron, copper, zinc, potassium, and magnesium, and the vitamins thiamine, riboflavin, and niacin (Salunkhe et al, 1985). A nutritionally complete range of amino acids are supplied when lysine-rich legumes are complimented with methionine and cystine replete grain cereals (Kay, 1979). In addition to providing human dietary sustenance, the cultivation of grain legumes provides an important source of animal forage and enhances soil fertility and quality (Okigbo, 1978).

Economic loss

Bruchids are the primary pest of stored grain legumes. Through advantageous immigration and evolutionary selection, bruchids are now entrenched at every level in the pulse ecosystem: in the field, in farm and household storages, at processing sites, during local, regional and international commodity transportation, and in foreign storage (Pedersen, 1978). Silim (1994) lists estimates of economic loss attributed to bruchid infestation of stored grain legumes at 35% in Central America, 7-13% in South America, and as high as 73% in Kenya, while damage specifically to stored cowpeas has been estimated to range between 15-40% in northern Nigeria (Caswell, 1968). Labeyrie (1981) suggests that infestation levels of 80-100% may be routine in the common bean, cowpea, and pigeon pea, based on "direct investigations, in village shops, at local merchants' and mainly in markets and in peasants' cabins in Columbia as well as in Mexico, High Volta, Syria or in Guadeloupe". Caswell (1961) reports that a 2% infestation of cowpea by Cm will result in complete destruction of the commodity within several months of storage if left untreated.

Postharvest loss

Salunkhe et al (1985) defines postharvest loss in food legumes as: loss of commodity weight in the period between harvest and consumption; loss of nutrients in stored legumes; qualitative deterioration caused by contaminants or biochemical changes rendering legumes unfit for human

consumption; loss of seed viability; and loss as a result of physical damage. Unfortunately, bruchids can inflict all of these types of loss, principally through commodity consumption and contamination with frass and uric acid. Increasing levels of Ao infestation are known to be correlated with an increase in levels of nitrogen and uric acid, and a decrease in protein content in various grain legumes (Regnault-Roger et al, 1994). Contamination from uric acid, a common insect protein metabolite, is correlated with negative changes in legume nutritive composition, including increased fat acidity and decreased levels of various vitamins and essential amino acids (Salunkhe et al, 1985). Finally, bruchid infestation broaches the protective seed testa and provides a means of access for storage microorganisms and secondary insect pests (Tipples, 1995).

Bruchid control measures

Contact insecticides and fumigants

Curative rather than preventive measures are more frequently employed in both large and small-scale grain legume storages to contain bruchid damage below severe economic injury levels (Labeyrie, 1981), although technical and financial constraints significantly reduce the use of pesticides (Javaid et al, 1993). Commercially available chemicals most commonly used to control bruchid infestation in stored grain legumes include the contact insecticides pyrethrins, organophosphates, and carbamates, and fumigants (carbon disulfide, carbon tetrachloride, methyl bromide, ethylene dichloride, ethylene dibromide, chloropicrin, and phosphine) (Salunkhe et al, 1985). The usefulness of chemical control has been severely limited by: the prohibitive cost of the chemicals (Sode et al, 1995); inadequate training and consequent poor applications leading to insect resistance (Sriharen et al, 1990) and human health hazards (Annis et al, 1990); and rapid chemical dispersion and deterioration in rustic storages and under extreme environmental conditions (Taylor, 1978). Policies reflecting the increased public pressure to reduce and eventually eliminate dependence on pesticides have resulted in the probable non-reregistration of the commonly used surface dressing malathion (Higley at al, 1992) and the approaching international ban of the fumigant methyl bromide, a 1992 amendment to the Montreal Protocol of the Vienna

Convention which identified it as an ozone depleting substance (Anonymous, 1993). These events coupled with the high cost of developing and registering alternative pesticides that will have limited useful lifetimes dictated by pest resistance have significantly limited chemical control options in stored products.

Controlled/modified atmospheres

Controlling or modifying the atmosphere of storage facilities by altering normal atmospheric gas ratios (78% nitrogen, 21% oxygen, and 1% rare gases)(Peng, 1990) to high carbon dioxide or high nitrogen/low oxygen content is an effective method for treating infested stored products. Evidence suggests that the practice of hermetically sealing grain in storage vessels, thereby increasing carbon dioxide while decreasing oxygen to lethal levels by the respiration of the grain, to control pests and associated organisms may have been employed by the ancient Egyptians and continues today in Africa (White and Leesch, 1995). This method does not taint the treated commodity with persistent toxic residues characteristic of contact insecticides, but flavor deterioration may occur when carbonic acid is generated as a by-product of carbon dioxide reacting with the stored product (White and Leesch, 1995). The benefits of this control method are confounded by economic deterrents: the cost of application, length of treatment required for effective control, availability of an adequate supply of gas at the storage site, and the requirement for air-tight storage facilities (White and Leesch, 1995).

Physical treatments

Physical treatments have proven more successful in small-scale farm storage, probably because the methods and materials are relatively simple and low cost. These methods are characterized primarily by the adaptation of large-scale pest control techniques to locally-available materials and storage facilities. Reduction in bruchid infestation has been acheived using simple physical controls such as: storing pulses unthreshed in traditional granaries where the dry pod provides a physical barrier against oviposition by Cm (Caswell, 1974); sun drying (Rahman, 1990); storing beans above the 36°C thermal threshold for Ao larval survival and adult reproduction (Huignard, 1978); freezing (LeRoi et al, 1991); hanging small lots of beans in the protective smoke over the kitchen fire (van

Huis, 1991); and crushing eggs or perturbing oviposition by bean sieving (Silim, 1994) or bean tumbling (Quentin et al, 1991). The admixture of local dusts, smaller grains, wood ash or sand to stored legumes reduces the intergranular space and progressively limits the available area for generations to utilize, thereby causing autosterilization as a result of crowding, exhaustion of food, rise in local moisture and microbial overgrowth (Salunkhe et al, 1985).

Botanically-derived treatments

Topical treatment of grain legumes with various oils provides a fumigant effect on adult bruchids (Regnault-Roger and Hamraoui, 1994), a physical barrier to oviposition, a repellant to female Cm seeking oviposition sites (Daniel and Smith, 1991), and in some cases, provides a botanically-derived ovicide (Schoonhoven, 1978; Khaire et al, 1992). Mixing stored legumes with plant materials or preparations derived from local perennial mints, peppers (*Capsicum* sp.), or neem tree (*Azadirachta indica*), with antifeedant, repellant or insecticidal biorational properties has met with varying degrees of success (Weaver et al, 1992; Kayitare and Ntezurubanza, 1991; Ivbijaro and Agbaje, 1986; Tanzubil, 1991; and Baby, 1994). Lienard and Seck (1994) provide a comprehensive list of plant species and their mode of action for controlling Cm. Physical and botanical control measures are generally advantageous because they are locally available and affordable; however, most of the treatments described above could be as harmful to beneficial insects as they are to pest insects.

Resistant cultivars

In recent years priority has been given to the successful development of pest resistant strains of various grain legume species, particularly the cowpea, *Vigna unguiculata* (L.) Walp. and the common bean, *Phaseolus vulgaris* L. (Dobie, 1987). Plant resistance mechanisms take two forms: general defensive substances protecting against non-pest species, and pest-specific antimetabolic or toxic secondary metabolites (Gatehouse et al, 1990). Selection for naturally elevated levels, or genetic manipulation to enhance, antimetabolic plant defensive proteins in specific cultivars effectively renders those plants nutritionally inaccessible by inhibiting insect digestive protienase. Screening

programs to determine susceptibility to infestation of locally grown or commercially available legume landraces or varieties to locally collected strains of pest bruchids is useful in selecting resistant cultivars (Javaid et al, 1993). Additionally, selection can be made for mechanical resistance in the form of thick seedcoats impenetrable to hatching larvae and/or emerging adults (Horber, 1978).

BIOLOGICAL CONTROL

Introduction

The use of biological control agents to limit bruchid populations under both field and storage conditions would appear to circumvent many of the concerns and limitations of the control methods discussed above, particularly with reference to economic constraint, local availability and potential harm to human health and the environment. Biological control as a major component of integrated pest management is perhaps one of the few methods of insect control where costs can be directly offset by increased labor in the form of diligent sanitation, frequent monitoring of the crop or commodity, conscientious cultivation and storage practices, and application of all locally available, compatible control methods. Detractors claim that biological control is too sophisticated for many on farm storage situations, although a relatively simple method for discovering locally occurring natural enemies of bruchids is available. This consists of placing infested legumes in paper sacks, setting them out in the field (or storage facility) until parasitism can occur, then collecting and identifying the emerging non-bruchid insects. This method could in fact be used to establish an *in vivo* continuous mass culture of biological control agents on previously infested, essentially useless legumes (Hetz and Johnson, 1988). In the case of primitive storage in developing countries, classical biological control and inoculation/augmentation/inundation strategies are not generally feasible, but manipulation of the storage environment to conserve and enhance the number and abundance of natural enemy species is possible (van Huis et al, 1991).

History of biological control

The earliest known case of biological control of an agrarian pest dates from 900 A.D. Asia when predatory ants were placed on citrus trees

to attack Coleopteran and Lepidopteran pests (Sweetman, 1958). Classical biological control of field pests in which the natural enemies of imported pests are identified in the pests' indigenous environment and then cultured and released in the adopted habitat has become a common practice, although success varies greatly from case to case (Hall et al, 1980). The most renowned case of classical biological control was the discovery and release of the Australian vedalia or lady bird beetle, *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae), which successfully curtailed catastrophic damage to Californian citrus groves by the cottony-cushion scale, *Icerya purchasi* (Maskell) in the late 1880's (van den Bosch et al, 1982).

Biological control of stored-product pests

The limited knowledge and practice of biological control of storage pests contrasts sharply with the numerous successful discoveries and applications of biological control of field pests. Arbogast (1976) attributes the lack of research in this area in part to socio-economic practices and biases intolerant of any insect, beneficial or pest, in stored commodities. The reduction in market price/grade of stored commodities infested beyond the accepted and regulated number of insects or insect parts per bushel has historically discouraged the development and application of biological control to stored product protection, even if the entomophages can prevent actual commodity damage. In 1992, the U.S. Environmental Protection Agency granted an exemption for parasitic and predatory insects of stored product insects in bagged and bulk-stored raw grains and legumes from the requirement of a tolerance, the maximum allowable residue level in food (Federal Register, Vol. 57, No. 78). Most perception of the success or failure of natural enemies of bruchids is "based on observation and not experimental evidence," (Southgate, 1978). Though parasitoids from several families (Trichogrammatidae, Eupelmidae, Pteromalidae, Braconidae, Eulophidae, Torymidae, etc.) have been recorded in association with bruchids, both in the field and in storage (Prevett, 1961; deLuca, 1965; Steffan, 1981; Hetz and Johnson, 1988; van Huis, 1991; and Lienard and Seck, 1994), few studies have been undertaken to quantify their efficacy. Arbogast (1984) and Brower et al (1996) provide a comprehensive overview of the role of biological control

in stored products research to date.

Hymenopterous parasitoids of bruchids

Anisopteromalus calandrae (Howard)

The larval ectoparasitoid *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae), Ac, is classified as follows: Hymenoptera (order); Apocrita (suborder); Chalcidoidea (superfamily); Pteromalidae (family); Pteromalinae (subfamily); and Pteromalini (tribe), according to Hanson (1995). Adult Ac are described in Waterston (1921) under the synonym *Aplastamorpha vandinei* Tucker. Studies have indicated that field strains of Ac are 90-fold more resistant to malathion than the field strain of its host, the rice weevil, and significantly more resistant to chlorpyrifos-methyl and pirimiphos-methyl than either its host or the susceptible lab strain of Ac (Baker and Weaver, 1993), thus indicative of its suitability for stored products IPM.

The association of Ac with *Callosobruchus* spp. was noted in studies of immature Ac morphology (Chatterji, 1955) and field infestation of cowpea in Nigeria (Prevett, 1961). Subsequent ecological experimentation has demonstrated a linear relationship in Ac functional response to parasitism of Cc and that increased host egg density can be correlated with parasitoid searching success (Ryoo and Chun, 1993). It was determined that Ac exhibits Holling's Type II functional response to the third, fourth and pupal stages of Cm while increased host searching efficiency was not correlated with increased handling time (Heong, 1982). The efficiency of Ac parasitism of Zs increased significantly 16-18 days after larval emergence, indicating that a life-history refuge from parasitism exists for the egg and early instar larval stages of Zs and ultimately stabilizes the parasitoid-host system by ensuring synchrony between the host and parasitoid life cycles (Kistler, 1985). The developmental and reproductive biology and morphological descriptions of various stages of Ac on the bruchid host Cc are reported in Islam (1993).

***Pteromalus cerealellae* (Ashmead)**

Another pteromalid larval parasitoid, *Pteromalus cerealellae* (Ashmead), is classified as follows: Hymenoptera (order); Apocrita (suborder); Chalcidoidea (superfamily); Pteromalidae (family); Pteromalinae (subfamily); and *Pteromalus* (= *Habrocytus* Bouček (1988),

generic revision) (genus), according to Hanson (1995). All stages of Pc are described in Noble (1932). Pc, thought to be a monophagous parasitoid of the Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Fulton, 1933; Noble, 1932), was found to attack and successfully develop in twelve beetle species, including Cm. Percent reduction in number of emerging bruchid progeny in treated versus untreated Cm, Cc, and Ca infested legumes was 96.6, 66.9, and 10.2, respectively (Brower, 1991).

***Uscana* species egg parasitoids**

Recent studies in the biological control of bruchids has centered on *Uscana* spp. (Hymenoptera: Trichogrammatidae) egg parasitoids. There are nine *Uscana* species known to attack bruchid eggs (van Huis et al, 1991).

The hemipterous predator *Xylocoris flavipes* (Reuter)

Xylocoris flavipes (Reuter) is a generalist predator of stored-product insects. Xf is classified as Hemiptera (order); Gymnocerata (suborder); Cimicoidea (superfamily); Anthocoridae (family); Lyctocorinae (subfamily); Xylocorini (tribe); *Xylocoris* (genus); *Arrostelus* (subgenus), according to Henry (1988). Xf is commonly known as the warehouse pirate bug. Xf is cosmopolitan in distribution (Henry, 1988; Gross, 1954) and is commonly reported from storage habitats (Jay et al, 1968) in association with its prey, the eggs, larvae and pupae of pest lepidoptera and coleoptera (Arbogast, 1978) infesting various stored products (Awadallah and Tawfik, 1972). Other anthocorids known to be effective biological control agents include *Anthocoris confusus* and *Anthocoris nemorum*, which attack the sycamore aphid, *Drepanosiphum platanoides* (Hill, 1957 and 1968; Dixon and Russell, 1972); and *Lyctocoris campestris*, another generalist predator of stored product pests (Parajulee et al, 1994). Descriptions of the various life stages of Xf appear in Gross (1954), Arbogast et al, (1971), and Awadallah and Tawfik (1972). The external morphological character distinguishing the sexes is the shape of the apex of the abdomen (♂ notched on the left side of segments 8 and 9; ♀ bilaterally symmetrical) (Gross, 1954). Both brachypterous and macropterous forms occur in Xf, although the short-winged form was found to be most common in a sampled population (Arbogast, 1978).

***Xf* biology**

Xf hatches from ellipsoidal eggs 0.67 mm long x 0.26 mm diameter,

usually 4-5 days after being laid randomly throughout stored grains and legumes and related detritus (Arbogast, 1978). The nymph passes through incomplete metamorphic development consisting of five instars (Arbogast et al, 1971). Developmental maturity is reached in 14-35 days and is highly influenced by temperature (protracted at 20°C, most rapid at 30-35°C) and to a lesser degree, relative humidity (Arbogast, 1975). Extremes of both humidity and temperature influence fecundity (Arbogast, 1975; Awadallah and Tawfik, 1972). The species would not be suited to use in unheated storage facilities during winter in temperate zones because Xf imagos are cold sensitive: eggs of Xf held at 5°C for 4 days, or 10 or 15°C for 16 days will not hatch and survivorship of second instar nymphs from eggs exposed to shorter periods of low temperature was significantly reduced (Press et al, 1976). Population growth rate is optimal at environmental conditions between 29-31°C and 60-70% relative humidity (Arbogast, 1978). Adults vary in size from 1.93-2.55 mm, the females being slightly larger. Mating begins on the same day as adult emergence (Awadallah and Tawfik, 1972). Insemination is extragenital traumatic and essential to reproduction because seminal stimulus is required for normal egg development (Arbogast, 1978). Mean adult life span is 21.6 days, and in a mean oviposition period of 17.5 days the average female Xf will lay 41.6 eggs (Arbogast, 1975). Xf emits a highly volatile, odorous secretion possibly for defensive purposes which is comprised of four monoterpene alcohols: linalool, α-terpineol, nerol, and geraniol, which individually and combined vary in insecticidal fumigant activity (Phillips et al, 1995).

***Xf* predatory attributes and ecology**

Xf exhibits several desirable qualities for effective predation. Xf is efficient at searching out scant prey distributed within bulks of unprocessed stored commodities. Reduced pest suppression could be directly correlated with a reduction in particle size of the medium searched (LeCato, 1975; Press et al, 1978). In a small scale test, the population growth of the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), was reduced by 95% or more, even when as few as five pairs and as many as thirty pairs of Xf were introduced into sealed 5-gallon drums containing 32-liter lots of shelled corn infested with 20

pairs of Os (Arbogast, 1976). The results of large scale warehouse tests indicate that Xf is well suited to the role of biotic pesticide for prophylactically disinfesting emptied storage bins and warehouses of residual populations of stored product pests which threaten to infest fresh commodities when they are first brought in from harvest (Brower and Press, 1992; Brower and Mullen, 1990; Press et al, 1975; LeCato et al, 1977). When presented with a choice of prey, Xf will generally attack the most easily subdued or penetrable prey (LeCato and Davis, 1973; LeCato, 1976) depending on prey size, vestiture, degree of sclerotization, or defensive behavior (Arbogast, 1978), but it will persistently attack known non-preferred prey until feeding can successfully occur to avoid starvation when preferred prey is not available (LeCato, 1976). Although 95% of adult Xf observed died after 120 hours of starvation, starvation for a period up to 96 hours did not significantly reduce the percentage or number of prey killed (LeCato, 1976). Xf exhibited a propensity toward cannibalism when prey was absent, theoretically a survival strategy for the perpetuation of a local population until a new influx of prey could occur (Arbogast, 1979). Xf requires few prey to survive (LeCato and Collins, 1976), but adapts to a surfeit of prey by increasing its rate of predation as the number of available prey increases, as exhibited in its functional response to the Angoumois grain moth, *Sitotroga cerealellae* (Olivier) (LeCato and Arbogast, 1979). Xf can be successfully utilized in concert with other natural enemies to achieve increased control efficacy without introducing chemical controls, aiding in the conservation of naturally-occurring biological control agents and reducing the use of pesticides on known resistant pest populations. Keever et al (1986) found that the combined biocontrol treatment of Xf paired with the larval parasitoid *Bracon hebator* Say (Hymenoptera: Braconidae) was more effective than a conventional malathion chemical control program in controlling malathion resistant pests infesting commercial warehouses of farmers stock peanuts, although Xf has been observed to predate upon larval stages of Bh (Press et al, 1974). In addition, Baker and Arbogast (1995) have determined that the field strain of Xf is 31-33 fold (♀ and ♂, respectively) resistant to malathion relative to the LD_{50} of the susceptible laboratory strain of the predator, and attribute resistance

development to detoxification of malathion by an unidentified carboxylesterase.

Predators associated with storage bruchids

Although deLuca's *Catalogue des Metozoaires Parasites et Predateurs de Bruchides (Coleoptera)* (1965) lists staphalinids, mantids, tachinids, and reduviids among bruchid predators, no studies have been published thus far documenting the rate of predation or other parameters of predatory efficacy of any beneficial species on bruchids infesting stored grain legumes. Specimens of Xf were recovered from imported mixed grains, rice, and *Vigna* (cowpea) seeds by the U.S. Department of Agriculture Insect Identification and Parasite Introduction Research Branch, Beltsville, MD (Jay et al, 1968). Arbogast (1978) reports that researchers found Xf to be ineffective against pest species which develop within seeds, including Cm. Conversely, El-Nahal et al (1985) reports a specific association of Xf and two species of Bruchidae, *Bruchidius incarnatus* Boh. and *Bruchus rufimanus* Boh., infesting Egyptian stores of horse or broad beans, *Vica faba* L. According to Kay (1979), broad beans are one of the most widely disseminated and ancient food crops; having originated in the Near East, they are thought to be the only bean known in Europe in the pre-Columbian era, and have now spread to virtually all temperate and subtropical regions. It is therefore plausible that the association of Xf and pest bruchids in stored grain legumes has been long standing, if not fully understood.

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Connecting Statement I

Bruchids are the primary pests of stored grain legumes. Current methods for preventing or suppressing bruchid infestation of stored legumes are largely unsatisfactory and further investigation is merited to evaluate alternative pest management strategies. *Xylocoris flavipes* is well-documented as a generalist predator of stored-product pests but evaluation of predation on bruchids has not been reported. The immature stages of most bruchid species are inaccessible to predation, and the adults are significantly larger than *X. flavipes*. The functional response of the predator to adult bruchids, and to the egg and neonate larval stages of *Acanthoscelides obtectus*, was measured to establish the occurrence and rate of predation (Section II).

Section II: Functional Response

Title

SUPPRESSION OF BRUCHIDS INFESTING STORED GRAIN LEGUMES WITH THE PREDATORY BUG *XYLOCORIS FLAVIPES* (REUTER) (HEMIPTERA: ANTHOCORIDAE): I. FUNCTIONAL RESPONSE TO ADULT AND IMMATURE BRUCHID STAGES

Abstract

Economic and material constraints render biological control one of few potential management tools to offset postharvest losses due to bruchid infestation of stored grain legumes, a primary source of human dietary protein in developing countries. The functional response of the cosmopolitan predatory bug *X. flavipes* to the adult stage of five economically significant bruchid species, including *Acanthoscelides obtectus*, *Callosobruchus analis*, *C. chinensis*, *C. maculatus*, and *Zabrotes subfasciatus* was Type II density dependent. Data were fit using Holling's disk equation. A negative correlation exists between mean pest species body weight and rate of predation. Female predators killed more adult bruchids than their male counterparts. *X. flavipes* potential to suppress *A. obtectus* populations was greatest because the eggs and neonate larvae are readily accessible. Mean predator kill of *A. obtectus* immature stages was 40 first instar larvae or 10-20 eggs per 24 hr interval. Further investigation of the biological control potential of *X. flavipes* against pest Bruchidae is merited because of the ability of the predator to kill adult stages of all prey species evaluated.

Introduction

Grain legumes, also known generically as pulses or dried beans, are often the only source of affordable and accessible dietary protein for the inhabitants of many developing countries (Smartt, 1990). Bruchids, the seed beetles, are responsible for the greatest postharvest loss to stored grain legumes, directly by consuming the resource and secondarily by qualitative deteriorization of the commodity and reduced seed stock viability (Southgate, 1979; Salunkhe et al, 1985). The economically significant bruchid species examined in the present study include *Callosobruchus analis*, *C. chinensis*, *C. maculatus*, *Acanthoscelides obtectus*, and *Zabrotes subfasciatus*. With the exception of *A. obtectus*, all species cement oviposited eggs onto the outer testa of the host seed with a protective coating and hatching larvae subsequently bore directly into the seed where all pre-eclosion development occurs. Therefore, predation on most bruchid species is limited to the adult stage. *A. obtectus* oviposits randomly among the seeds and the highly vulnerable eggs and first instar larvae are extremely susceptible to mortality by predation and dessication until the larva can enter a host seed (Howe and Currie, 1964).

Xylocoris flavipes (Reuter) (Hemiptera: Anthocoridae) is a predator of multiple species (Arbogast, 1978) and stages of stored-product pests, although it is most successful against small-sized, externally-developing prey, particularly the accessible eggs and early larval stages that are neither heavily sclerotized nor overly hirsute (LeCato and Davis, 1973). The objectives of this study were to ascertain if *X. flavipes* can successfully prey upon the adult stages of the bruchid species listed above, and to quantify the upper limit of immature *A. obtectus* prey that can be killed by the predator. The predator is known to readily attempt and increasingly persist in predation of non-preferred prey species/stages when faced with starvation if preferred prey are not available (LeCato, 1976). Observations made in preliminary experimentation confirmed that *X. flavipes* could successfully subdue and feed upon adults of all bruchid species examined here even though the prey were significantly larger in size than the predator (Sing, unpublished). The functional response of *X.*

flavipes against the eggs and early instar larvae of the Angoumois grain moth, *Sitotroga cerealella* indicated a preference for larval prey, which the authors surmised was typical of an obligate predator's attraction to the movement and size of the prey (LeCato and Arbogast, 1979). *X. flavipes* rate of predation on 'difficult' adult versus 'easy' egg or 'stimulating' larval prey will be compared.

Materials and methods

All bruchid species were maintained in continuous culture and experiments performed under identical environmental conditions of 12:12 hr scotophase:photophase and 29 ± 2°C, 65 ± 5% R.H. Cultures of *A. obtectus*, *Z. subfasciatus*, *C. analis*, and *C. chinensis* were started in 1981 with stock received from the Pest Infestation Control Laboratory, Slough, Bucks, England and maintained in continuous culture at the Stored-Product Insects Research and Development Laboratory, Savannah, GA, USA. *C. maculatus* were obtained from a continuous culture which originated in Fresno, CA. The contents of culture jars were sifted using a U.S. Standard #6 sieve 24 hr after initial sifting to provide 0-24 hr post emergence experimental subjects. *A. obtectus* eggs used in the present study were 0-24 hr, gathered from oviposition jars fitted with screening platforms allowing eggs to drop through to petri dishes positioned below. *A. obtectus* larvae were collected from hatching arenas where fresh eggs were placed until hatching occured, approximately 96-120 hr after oviposition.

A continuous culture of *X. flavipes* originating from specimens collected in 1977 from a purposely-infested experimental warehouse facility at the Stored-Product Insects Research and Development Laboratory, Savannah, GA, USA was maintained under the environmental conditions stated above, and reared in 3.78-liter glass jars provided with Hexcell® paperboard harborage and previously-frozen *Plodia interpunctella* (Hübner) eggs as a food source. Continuous culture jars were cleared of all adult predators and experimental subjects collected from a pool of adults emerging 0-6 days after initial sorting. Subjects were sexed according to Arbogast et al (1971) and retained individually in gelatin capsules.

Experimental arenas consisted of 9.0 cm glass petri dishes treated on the sides with liquid Teflon® and allowed to cure for 24 hr after application in a fume hood. Interior arena bases were fitted with a fine mesh nylon fabric circle to provide footing. Both measures were taken to ensure that the prey remained accessible to *X. flavipes*, which experienced difficulty walking and climbing on the smooth glass surfaces of the arenas. Five replicates of 5, 10, 15, or 20 adult prey individuals per arena were set up and exposed to one of three treatments: control; one male predator; or one female predator. Arenas were checked at three 24-hr intervals and each time the number of dead prey were recorded, and dead prey were replaced with live so that the number of potential live prey remained constant. *A. obtectus* egg and larvae prey densities were 5, 10, 15, 20, or 50 individuals and experiments followed the protocol described above, except that experiments were terminated after 24 hr. All experiments were performed twice.

A general linear models procedure PROC GLM, (SAS Institute, 1988) was used to discriminate contributions of predator sex and replicate experiment to total variation observed in the numbers of bruchids killed. In general, predator sex was significant in this analysis, so Holling's Type II curvilinear functional response equation, PREY KILLED = (EXPOSURE TIME * PREY AVAILABLE * RATE OF DISCOVERY/(1+ (RATE OF DISCOVERY * HANDLING TIME * PREY AVAILABLE)), was fit to the number of adults for each bruchid species and *A. obtectus* eggs and larvae killed for each predator sex (and replicate experiment, where applicable), using PROC NLIN (SAS Institute, 1988). The exposure time for the prey in all of these experiments was 24 hr, so this quantity was a constant for all fitted equations. Each model was evaluated for size of the regression F statistic and the lack-of-fit F statistic, as well as the approximate variation explained (r^2).

Results

All prey were attacked in a density dependent fashion by both sexes in all experiments (Table 1), with the functional response generally being adequately described by Holling's disk equation (Figure 1 & 2; Table 2).

The immature stages of *A. obtectus* were actively preyed upon by both

sexes of *X. flavipes* (Fig. 1). Both sexes preyed equally on the active neonate larvae. It is not apparent that the upper limit of predation was reached on this stage in these experiments because the data indicate that a small quantity of prey consistently remained untouched at most densities examined. At the highest larval prey density of 50, nearly 40 prey were killed by the predator in a 24 hr interval (Fig. 1B).

A. obtectus eggs were also heavily predated by female *X. flavipes*, less so by the male predators (Fig. 1A). The upper limit of predation by both sexes was approximated in this study, with 20 eggs per female and 10 eggs per male being the average kill for a 24 hr interval.

The large adults of all five bruchid species were also predated, at low levels, by both sexes (Fig. 2). A weak, but significant functional response was evident for both sexes, although it was more pronounced for female predators (Fig. 2 - filled circles). In general, at a density of 8 prey per arena, a female predator killed an average of greater than 1 prey per 24 hr interval, whereas males killed less than 1 prey in the same time interval.

Two data sets showed a weak but significant lack-of-fit (at α = 0.05) with Holling's disk equation. These were for *C. analis* adult prey being predated by the female predator (Fig. 2B; Table 2) and *C. maculatus* adults being predated by the male predator (Fig. 2D; Table 2). The lines were plotted because there are no better fitting biologically plausible models and the problem actually rests with the variability in the low predation rates observed.

The instantaneous rates of prey discovery were quite consistent for all prey, but handling times varied from 12 minutes per *A. obtectus* larvae by either predator sex to greater than 40 hr for adults of *A. obtectus* and *Z. subfasciatus* being predated by the male predator (Table 2).

Discussion

Although most predators characteristically attack the largest available prey, that prey is generally always smaller in body size than the predator, with the exception of those predatory arthropods that increase maximum prey size beyond their own body size by ambushing prey and subduing it with offensive venoms (Sabelis, 1992). The results of the

present study indicate that *X. flavipes* is capable of low level but fairly consistent success in killing much larger adult bruchid prey. Stimulated *X. flavipes* direct a scent-gland exudate thought to be defensive in nature over a wide area (Remold, 1963). On closer examination, the terpene constituents of this secretion, particularly α-terpineol and linalool, were found to have significant toxic activity against adult *Tribolium castaneum* (Herbst) and *Oryzaephilus surinamensis* (L.), the possible mode of toxicity being competitive inhibition of acetylcholinesterase (Phillips et al, 1995). Furthermore, the rapid liquifaction of large prey after attack by *X. flavipes* (Sing, unpublished) suggests that the predator utilizes an enzymatic salivary venom for extra-oral digestion, a common strategy of predaceous arthropods preying on large prey with intractable cuticles (Cohen, 1995). Finally, the low level of *X. flavipes* predation on adult bruchids can be explained not only by the greater challenge to subdue large prey, but is also correlated with daily ingestion rate and gut capacity; the nutritional resources of large prey generally exceed the daily food requirements of small predators (Peters, 1983). Predation of additional adult bruchids in experimental arenas may also be reduced by return feeding to readily apparent previous kills.

X. flavipes killed significantly more 'stimulating' larval prey than 'easy' egg prey, reiterating the results of LeCato and Arbogast (1979) with the prey species *Sitotroga cerealella* (Olivier) which suggested that *X. flavipes* is an obligate predator. The functional response of the predator to both immature stages of *A. obtectus* indicates that *X. flavipes* has potential as a biological control agent of this particular bruchid species. Predation on adult *A. obtectus* may have been confounded by the presence of eggs freshly oviposited by the experimental subjects. The potential of a 'knock down' or disorientation effect from the scent-gland secretion combined with the catastrophic disruption of neuro-muscular function (Blum, 1981) caused by the injection of salivary venom could account for the predator's ability to kill the comparatively more 'difficult' adult bruchid prey. Howe and Currie (1964) list the mean body weights of all bruchid species discussed here; results of the current study indicate that highest rate of predation occurred with the lightest species and decreased with increasing mean body weight of the prey

species. Additionally, observation of predator interaction with mated pairs of bruchid prey indicated a high level of mating and oviposition disruption, and opportunistic predation on bruchids engaged in copulation (Sing, unpublished), indicates that presence of *X. flavipes* in the bruchid - grain legume complex may significantly impact prey populations.

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Table 1. General linear model for contribution of predator sex and replicate experiment to total variation observed in functional response of *X. flavipes* to bruchid prey.

Source	df	F	Pr > F
	A. obtectus egg stage		
sex	1, 116	12.84	<0.01
experiment	1, 116	1.23	0.27
	A. obtectus larval stage		
sex	1, 116	0.00	0.99
experiment	1, 116	0.43	0.52
	A. obtectus adults		
sex	1, 76	10.44	<0.01
experiment	1, 76	2.61	0.11
	C. analis adults		
sex	1, 76	15.09	<0.01
experiment	1, 76	2.77	0.10
	C. chinensis adults		
sex	1, 76	35.07	<0.01
experiment	1, 76	0.84	0.36
	C. maculatus adults		
sex	1, 76	5.40	0.02
experiment	1, 76	1.42	0.24

Z. subfasciatus adults

sex	1, 76	27.45	<0.01
experiment	1, 76	7.01	0.01

Table 2. Parameter values and regression statistics for Holling's Type II functional response equation for *X. flavipes* predating on bruchid prey.

IRD[a]	HT[b]	F_{reg}[c]	F_L[d]	r^2	% max. r^2 possible[e]
	A. obtectus egg prey, female predator				
0.0457 ± 0.0046	0.5318 ± 0.0695	494.63	1.18	0.02	100
	A. obtectus egg prey, male predator				
0.0597 ± 0.0147	1.8875 ± 0.2331	167.41	0.75	0.47	100
	A. obtectus larval prey, both predator sexes				
0.0466 ± 0.0022	0.1830 ± 0.0260	2015.07	0.64	0.93	100
	A. obtectus adult prey, female predator				
0.0463 ± 0.0726	27.3858 ± 8.0833	33.43	0.07	0.01	100
	A. obtectus adult prey, male predator				
0.0219 ± 0.0316	48.7095 ± 15.9528	29.39	1.18	0.02	100

C. analis adult prey, female predator

0.0326 ± 0.0117	7.4748 ± 2.0196	130.51	3.53	0.30	100

C. analis adult prey, male predator

0.0214 ± 0.0130	13.0762 ± 5.3589	48.74	1.98	0.09	100

C. chinensis adult prey, female predator

0.0269 ± 0.0104	8.8898 ± 2.6262	111.43	0.05	0.24	100

C. chinensis adult prey, male predator

0.0244 ± 0.0327	31.2339 ± 12.5484	22.87	0.29	0.02	95

C. maculatus adult prey, female predator

0.0066 ± 0.0024	-4.0937 ± 7.6857	59.36	0.56	0.36	100

C. maculatus adult prey, male predator

0.0156 ± 0.0142	24.3977 ± 11.5420	26.99	4.83	0.03	100

Z. subfasciatus adult prey, female predator, experiment 1

0.0335 ± 0.0394	14.6164 ± 7.3133	19.54	2.74	0.08	100

Z. subfasciatus adult prey, female predator, experiment 2

0.0217 ± 0.0118	6.3438 ± 4.2254	44.32	0.72	0.28	100

Z. subfasciatus adult prey, male predator, experiment 1

-0.0396 ± 0.1894	87.0010 ± 43.6867	7.39	0.07	0.00	100

Z. subfasciatus adult prey, male predator, experiment 2

0.0060 ± 0.0032	0.3627 ± 12.9664	30.75	0.29	0.32	100

[a]IRD= Instantaneous rate of discovery.

[b]HT= Handling time.

[c]$F_{reg}=(SS_{Regression}/df_{Regression})\div(SS_{Residual}/df_{Residual})$. All values of F are significant at $P\leq 0.05$ with the exception of *C. maculatus* on garbanzo beans, Exp. #2, at 120 hr (F significant at $P \leq .25$; and *Z. subfasciatus* on white navy beans, Exp. #1, at 120 hr (F significant at $P \leq .5$).

[d]$F_L=(SS_{Lack\text{-}Of\text{-}Fit}/df_{Lack\text{-}Of\text{-}Fit})\div(SS_{Pure\ Error}/df_{Pure\ Error})$. None of the reported values of F indicate a signigicant lack of fit at $P \leq 0.05$.

[e]% of maximum r^2 possible=$[((SS_{Regression}-SS_{Total}+SS_{Corrected\ Total})/(SS_{Corrected\ Total})\div((SS_{Corrected\ Total}-SS_{Pure\ Error})/SS_{Corrected\ Total})\times 100\%]$

Figure 1. Predation on immature stages of *A. obtectus* by *X. flavipes* adults as a function of prey density. (A) predation of eggs in a 24 hr interval, ●-● female predator, O-O male predator. (B) predation of neonate larvae by both sexes. Lines plotted are Holling's disk equation fitted to the data.

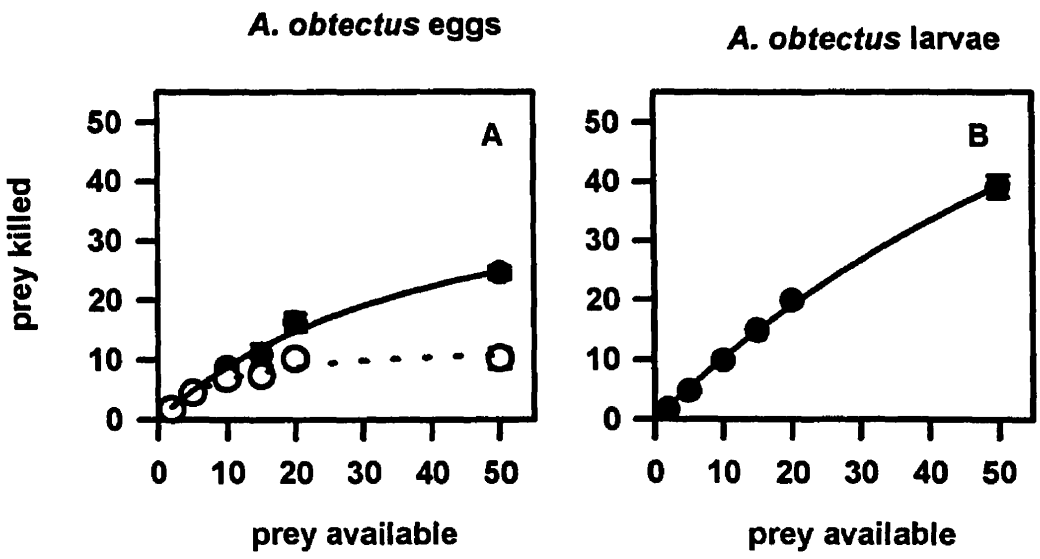
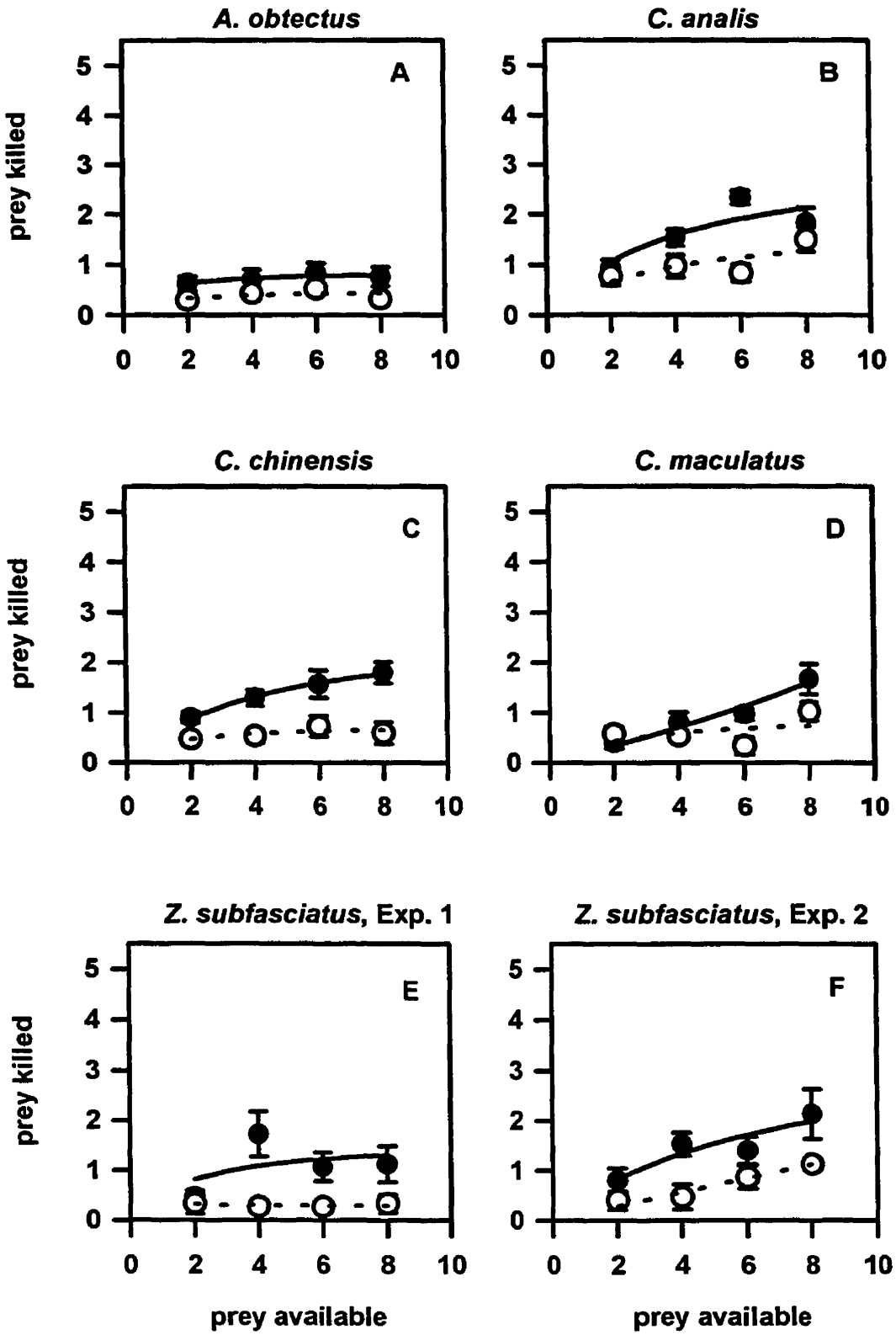

A. obtectus eggs
A. obtectus larvae
prey killed
prey available
prey available
A
B
0
10
20
30
40
50

Figure 2. Mean predation of adult stages of bruchids by *X. flavipes* as a function of prey density. Female predator ●-●, male predator O-O. Bruchid prey species: (A) *A. obtectus*, (B) *C. analis*, (C) *C. chinensis*, (D) *C. maculatus*, (E) *Z. subfasciatus* - experiment 1, and (F) *Z. subfasciatus* - experiment 2. Plotted lines are Holling's disk equation fitted to the data.


A. obtectus
C. analis
C. chinensis
C. maculatus
Z. subfasciatus, Exp. 1
Z. subfasciatus, Exp. 2
A
B
C
D
E
F
prey killed
prey available
0
1
2
3
4
5
0
2
4
6
8
10

Connecting Statement II

The functional response of *X. flavipes* to bruchid prey, low but consistent on the adults of all species examined, and much higher on the eggs and neonate larvae of *A. obtectus*, established and quantified the rate of predation on these species. Useful application of this predator to the problem of bruchid infestation of stored grain legumes would entail the development of an effective treatment protocol. Further experimentation was undertaken to measure the effects of predator density and time elapsed between legume infestation and predator addition on levels of emerging bruchid progeny (Section III).

Section III: Population Interactions

Title

SUPPRESSION OF BRUCHIDS INFESTING STORED GRAIN LEGUMES WITH THE PREDATORY BUG *XYLOCORIS FLAVIPES* (REUTER)(HEMIPTERA: ANTHOCORIDAE): II. INFLUENCE OF TIME OF PREDATOR ADDITION AND PREDATOR DENSITY.

Abstract

The influence of both elapsed time between initial infestation and introduction of predators, and predator density, were determined for suppression of bruchids infesting stored grain legumes by *Xylocoris flavipes* (Reuter)(Hemiptera: Anthocoridae). Efficacy of malathion resistant and susceptible strains of the predator were compared. Suppression of *Acanthoscelides obtectus* approached eradication with all predator treatments, while at the most effective treatment time and predator density (0 h; 5 predator pairs) for all other bruchid species (*Callosobruchus analis*, *C. chinensis*, and *C. maculatus*, and *Zabrotes subfasciatus*), reduction in emerging F_1 bruchids surpassed 50%, as compared to the untreated arenas. The predator addition time of 0 h, when predators were added to experimental arenas simultaneously with the pest species was the universally most efficacious treatment time. Predator density was less influential overall; when *X. flavipes* was added 24 or 120 h after initial bruchid infestation, maximum suppression was achieved at approximately 2 predator pairs and not significantly improved upon with increased predator density. Malathion-resistant field-collected strains of *X. flavipes* were found to be slightly less effective in the suppression of *C. chinensis*, *C. maculatus*, and *Zabrotes subfasciatus* than the malathion-susceptible strain of the predator.

Introduction

Xylocoris flavipes (Reuter) (Hemiptera: Anthocoridae) is a cosmopolitan, (Gross, 1954) generalist predator of coleopteran and lepidopteran stored- product pests (Arbogast, 1978). Evaluation of the biocontrol efficacy of *X. flavipes* against prey species under a variety of environmental conditions is well-documented (Brower et al, 1995), although experimentation with bruchid, or seed beetle, prey is limited. Researchers have established that *X. flavipes* is ineffective in controlling the immature stages of pest species such as bruchids which typically develop within seeds (Arbogast, 1978). However, a specific association of the predator and two species of bruchid, *Bruchidius incarnatus* Boh. and *Bruchus rufimanus* Boh. infesting Egyptian stores of horse or broad beans, *Vica faba* L., has been recorded (El-Nahal et al, 1985), as has the recovery of *X. flavipes* specimens from imported cowpea, *Vigna unguiculata*, by the U.S. Department of Agriculture Insect Identification and Parasite Introduction Research Branch, Beltsville, MD (Jay et al, 1968).

The results of preliminary experimental observations indicate that although *X. flavipes* is more than 50% smaller in body size than the smallest of its bruchid prey, the predator was capable of subduing and killing adult individuals of both sexes of five species of New and Old World Bruchidae, including *Zabrotes subfaciatus*, *Acanthoscelides obtectus*, *Callosobruchus maculatus*, *C. analis*, and *C. chinensis* (Sing, unpublished). Furthermore, *X. flavipes* significantly disrupted bruchid mating and oviposition (Sing, unpublished). The results reported here reflect an expanded inquiry into the influence on *X. flavipes* efficacy of bruchid species, bruchid host seed, predator density, and interval between bruchid infestation of the grain legumes and the time predators were added . Bruchid species size and possible defensive strategies, phytochemical incompatibility, predator competition and searching efficiency, and bruchid oviposition patterns were factors possibly impeding the effectiveness of the predator.

A subsequent study was undertaken to determine if a recently field-collected pesticide resistant strain of *X. flavipes* was more or less effective in controlling selected bruchid species than a pesticide

susceptible strain of the predator which had been maintained as a continuous laboratory culture for more than 20 years. Sustained malathion resistance, attributed to detoxification by an unidentified carboxylesterase (Baker and Arbogast, 1995), could result in reduced predatory efficacy, a possible manifestation of the fitness cost of resistance (Croft, 1990).

Materials and methods

All bruchid species were maintained in continuous culture and experiments performed under identical environmental conditions of 12:12 hr scotophase:photophase and 29 ± 2°C, 65 ± 5% R.h. Cultures of *A. obtectus*, *Z. subfasciatus*, *C. analis*, and *C. chinensis* were started with insects received in 1981 from the Pest Infestation Control Laboratory, Slough, Bucks, England and maintained in continuous culture at the Stored-Product Insects Research and Development Laboratory, Savannah, GA, USA. *C. maculatus* were from a continuous culture obtained from Fresno, CA. Bruchid host grain legumes were purchased locally in bulk 11.4 kg bags, held below 0° C for at least two weeks to ensure disinfestation, then acclimated under culture/experimental conditions in 0.95-liter Mason jars until the legumes reached environmental equilibrium, usually after one week. Equilibration was verified by repeated dry weight determination of grain legume moisture content. Experimental subjects were collected from culture jars which had been sifted through a U.S. number 6 standard sieve 24 hr previously to ensure that all individuals were 0-24 hr post emergence from the host seed. Individuals were sexed according to authoritative keys (Halstead, 1963; Southgate et al, 1957; Southgate, 1958), collected and retained in groups of five mated pairs in 18.3 ml plastic shell vials (7 cm h x 2.5 cm i.d.).

The pesticide-susceptible laboratory strain of *X. flavipes* originated from specimens collected in 1977 from a purposely-infested experimental warehouse facility at the Stored-Product Insects Research and Development Laboratory, Savannah, GA, USA. The malathion resistant strain was collected from infested farm stored shelled corn in Blackville, SC, USA, and resistant status established by Baker and Arbogast (1995). Both strains of the predator were maintained under the environmental conditions

stated above, and reared in 3.78-liter glass jars provided with Hexcell® paperboard harborage and previously-frozen *Plodia interpunctella* (Hübner) eggs as a food source. Culture jars were cleared of all adult predators and experimental subjects were collected from a pool of adults emerging 0-6 days after initial sorting. Subjects were sexed according to Arbogast (1978) and retained individually in gelatin capsules.

Experimental arenas consisted of half pint Mason jars filled with 100g of grain legumes. Five newly emerged (0-24 hr) mated pairs of bruchids were added to each of five replicate arenas per treatment. Predator density treatments of 0, 1, 2, 3, and 5 mated pairs of 0-6 day old adult *X. flavipes* were added to arenas at three different predator introduction intervals: 0, 24, and 120 hr to total 75 arenas per bruchid species/grain legume type. All experiments were performed twice. Each experiment was terminated according to a formula based on known adult emergence (from the continuous culture) of the first individuals of a bruchid species: arenas were held at experimental conditions for twice the approximate period to onset of adult emergence minus 10 days to ensure that only the F_1 generation was counted. Arenas were then frozen for two weeks, then contents were sifted through a series of sieves and bruchid numbers recorded.

In comparing biocontrol efficacy of pesticide resistant to pesticide susceptible predators, arenas consisting of 0.24-liter Mason jars were filled with 100 g blackeyed peas. Five newly-emerged (0-48 hr) mated pairs of bruchids were added to each treatment arena at the same time as five (0-7 day old) mated pairs of predators. Five replicate arenas were set up for each treatment: pesticide resistant predator; pesticide susceptible predator and control - no predator for three bruchid species (*C. chinensis*, *C. maculatus*, and *Z. subfasciatus*), for a total of 45 arenas. The experiment was performed twice; both experiments were terminated and data collected according to procedures described above.

Data were adjusted to subtract the number of parental adults from the total recorded for each arena. Analysis of variance (Proc ANOVA, SAS Institute, 1988) was used to assess contribution of experiment, predator number and time of predator addition to the total variation observed for each bruchid/legume combination. Most factors were significant for each

bruchid/legume system, so individual regression equations were generally fitted to each bruchid/commodity/experiment combination, and for each time of predator addition, using Table Curve 2D curve-fitting software (Jandel Scientific, San Rafael, CA). Selected equations were evaluated for percentage of variation explained (r^2) and for lack-of-fit, after initial sorting by F-statistic to provide simple equations that described the data well. The pattern and magnitude of residuals was also scrutinized.

For the predator strain experiment, replicate (1 or 2) and predator treatment (resistant, susceptible, or none) were assessed for contribution to the total variation observed in F_1 numbers for each bruchid species. There was a significant effect of experiment replicate for two of the bruchid species, so data are reported here separately for Experiments 1 and 2 for all species. Because a significant treatment effect was indicated by each ANOVA, each predator treatment was subsequently subjected to Dunnett's one-tailed t-tests to determine if the number of bruchid progeny was significantly lower in treated than in control arenas.

Results

The addition of *X. flavipes* to experimental arenas reduced adult emergence for most times of predator addition and for most predator densities on all bruchid/legume combinations evaluated here. *X. flavipes* suppression of *A. obtectus* approached eradication in all treatments; no variation in effect of time of predator addition or predator density was observed (Fig. 1, A and B). The most effective suppression of all other bruchid species resulted with predator addition at 0 h, when *X. flavipes* was added to experimental arenas simultaneously with parent bruchids. The least effective time for adding predators to arenas was at 120 h after initial bruchid infestation of the legumes (Fig. 1-3).

Similar suppression was observed for *C. analis* and *C. chinensis*; addition of *X. flavipes* was most effective when added at the same time as its prey but was nearly as effective when added 24 or 120 h later. The rapid approach of all plotted data to the asymptote indicates that predator density was a much less significant factor in suppression of these two species (Fig. 1, C-F).

Early predator addition to experimental arenas was key to

successful suppression of *C. maculatus* and *Z. subfasciatus*. Levels of suppression compared to the control were 60-70% when predator and prey were added at the same time, but became minimal when the predator was added 120 h later (Fig. 2 and 3). In particular, the 120 h treatments of *C. maculatus* on blackeyed peas, Experiment 2 (Fig. 2B), and *Z. subfasciatus* on white navy beans, Experiment 2 (Fig. 3D), were found by ANOVA to have a barely significant effect (see Table 2).

For all species evaluated, there was a significant reduction in the number of progeny emerging in the 1 pair predator density treatment compared to that of the controls, especially at 0 h. Otherwise, there was little difference in the effect of predator density other than for 5 pairs at 0 h, which was universally most effective. With the exception of *Z. subfasciatus*, prey suppression reached a maximum at two pairs of predators, regardless of when they were introduced, and increasing predator density further produced little additional suppression. When the predator was added at the same time as *Z. subfasciatus*, increased suppression was correlated clearly with increased predator density.

Reproduction of *X. flavipes* was observed in many 0 h treatment arenas, although it is not reported here (Sing, unpublished data). Significant predator population growth in treatment arenas was not possible because the numbers of prey available were low and of about the same age/stage. Because all species evaluated here other than *A. obtectus* develop inside seeds, predators introduced into experimental arenas were subjected to prolonged periods of starvation during the development of F_1 bruchid progeny once the nutritional resources from parental bruchids were exhausted.

Comparison of predatory efficacy of the pesticide-susceptible and pesticide-resistant strains of *X. flavipes* indicates that pesticide resistance is not a significant fitness cost. The pesticide-resistant predator strain was slightly less effective in bruchid suppression (Fig. 4).

Analysis of variance indicated that there was a significant effect of individual experiments within almost all bruchid/legume and bruchid/*X. flavipes* strain combinations (Tables 2 and 3), however, the figures (Figures 1-4) indicate that the treatment effects are quite similar. The

major difference observed is the relative number of F_1 progeny in control arenas. The relative rate of suppression by the predator is quite similar within experiments for each target species or for each predator strain.

Discussion

The results of this study indicate that *Xylocoris flavipes* can reduce the number of emerging bruchid progeny when applied at a variety of times and densities after initial bruchid infestation, but due to the inaccessiblity of the eggs and developing larvae of all species other than *A. obtectus*, is clearly most effective when it can begin to prey upon, or at least disturb mating and oviposition of the parental bruchids as soon as infestation occurs. Significant bruchid damage to stored grain legumes begins in most cases with low level field infestation which quickly grows to catastrophic proportions in the sheltered environs of storage facilities (Southgate, 1978; Labeyrie, 1981); this study reinforces the urgency of protecting stored legumes as soon as bruchid infestation is detected.

Previous studies with this predator have concluded that its best application lies in its demonstrated facility to prophylactically disinfest emptied storage facilities of residual populations of pest insect eggs and early instar larvae (Arbogast, 1978), a major source of contamination in newly stored commodities (Brower and Press, 1992; LeCato et al, 1977). This study suggests that *X. flavipes* could play a valuable role in preventive disinfestation of emptied legume storage facilities by reducing the threat of contamination to freshly stored legumes by residual storage populations. *X. flavipes'* ability to successfully attack large, scleritized prey when more accessible prey are not available (LeCato, 1976) was observed with all bruchid species evaluated here (Sing, unpublished) and appears to be reiterated in the results of this study. Because bruchids are typically the primary pest of stored legumes, *X. flavipes* predation on this family of pests would not be detracted from by the presence of more favored prey species.

The predatory efficacy of the malathion-resistant *X. flavipes* strain was only slightly lower than that of the susceptible strain. Such pesticide-resistant strains of natural enemies increase the options

available for commodity protection. Because the use of biological control agents typically eliminates or at least complicates the concurrent use of chemical controls, the decision is more often made to apply pesticides of a known efficacy, even when pest resistance is evident. This strain of *X. flavipes* has been shown to be very tolerant to malathion (Baker and Arbogast, 1995), and is potentially cross-resistant to other commonly used protectants.

The ability of this predator to reproduce successfully on bruchid prey was not tested in these experiments. In these experiments, the uniform age of parental bruchids represents a prey age structure which would ensure a life-history refuge from extinction, while at the same time forcing *X. flavipes* into starvation once all the parental bruchids had died and their cryptically-developing progeny had not yet emerged. However, under field conditions, the continual emergence of low numbers of bruchid adults from newly harvested legumes would provide sustained prey for predator population establishment.

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Table 3. Parameter values and statistical verification of regression equations analyzing data obtained from experimental treatments evaluating the effects of *Xylocoris flavipes* time added relative to bruchid infestation of commodity and predator density on number of emerging F_1 adult bruchids. Data and regressions are illustrated in Figs. 1-3.

Treatment time	Equation[a]	Parameter[b]	Value + S.E.	F_{Reg} [c]	F_L [d]	r^2	% of maximum r^2 possible[e]
			Acanthoscelides obtectus - Experiment 1 - Blackeyed peas				
0, 24, 120 hr			x = 0, y = 8.40 ± 2.91 x > 0, y = 0.03 ± 0.02	--	--	--	--
			Acanthoscelides obtectus - Experiment 2 - Blackeyed peas				
0, 24, 120 hr			x = 0, y = 41.07 ± 7.60 x > 0, y = 0.68 ± 0.18	--	--	--	--
			Acanthoscelides obtectus - Experiment 1 & 2 - White navy beans				
0, 24, 120 hr			x = 0, y = 17.33 ± 2.72 x > 0, y = 0.22 ± 0.08	--	--	--	--
			Callosobruchus analis - Experiment 1 - Blackeyed peas				
0 hr	1	a	81.2 ± 10.2	79.5	0.4	0.8	98.5
		b	189.7 ± 21.3				
24 hr	2	a	98.2 ± 6.6	105.5	1.9	0.9	99.4
		b	$1.4 \times 10^7 \pm 2.0 \times 10^{13}$				
		c	$-4.1 \pm 5.1 \times 10^5$				
		d	-0.4 ± 2.2				

120 hr	2	a	134.1 ± 2.4	485.1	0.7	1	99.9
		b	256.1 ± 1.7x10^5				
		c	0.05 ± 269.7				
		d	-0.2 ± 28.2				
			Callosobruchus analis - Experiment 2 - Blackeyed peas				
0 hr	3	a	78.7 ± 7.1	87.5	0.9	0.9	99
		b	174.0 ± 13.5				
		c	0.4 ± 0.2				
24 hr	3	a	95.7 ± 6.3	104.5	1.2	0.9	98.9
		b	161.3 ± 11.5				
		c	0.5 ± 0.1				
120 hr	3	a	147.0 ± 5.7	66.1	1.4	0.9	98
		b	126.1 ± 11.5				
		c	0.1 ± 5.9				
			Callosobruchus chinensis - Experiment 1 - Blackeyed peas				
0 hr	3	a	22.3 ± 4.9	152.7	0.2	0.9	99.9
		b	147.2 ± 8.6				
		c	0.6 ± 0.1				
24 hr	3	a	31.0 ± 5.3	91.2	0.4	0.9	99.6
		b	121.7 ± 9.3				
		c	0.6 ± 0.1				
120 hr	1	a	41.9 ± 5.8	84.3	0.1	0.8	99.7
		b	110.5 ± 12.0				

Callosobruchus chinensis - Experiment 2 - Blackeyed peas

0 hr	3	a	44.7 ± 5.2	47.6	0.2	0.8	99.6
		b	86.1 ± 9.1				
		c	0.6 ± 0.2				
24 hr	3	a	59.6 ± 5.4	39	0.1	0.8	99.8
		b	84.4 ± 9.8				
		c	0.5 ± 0.2				
120 hr	1	a	65.7 ± 5.7	24.3	0.8	0.5	90.8
		b	58.8 ± 11.9				

Callosobruchus maculatus - Experiment 1 - Blackeyed peas

0 hr	1	a	66.0 ± 9.6	80.5	0.5	0.8	98.1
		b	179.7 ± 20.0				
24 hr	1	a	83.1 ± 10.5	54	0.7	0.7	96.2
		b	160.8 ± 21.9				
120 hr	4	a	239.3 ± 11.2	14.6	0.2	0.4	96.5
		b	-15.3 ± 4.0				

Callosobruchus maculatus - Experiment 2 - Blackeyed peas

0 hr	1	a	94.4 ± 8.7	180.3	1.9	0.9	97.2
		b	243.8 ± 18.2				
24 hr	1	a	178.2 ± 8.7	73.4	1.3	0.8	95.3
		b	155.3 ± 18.1				
120 hr	1	a	299.9 ± 8.2	19.2	0.1	0.5	98.5
		b	74.6 ± 17.0				

Callosobruchus maculatus - Experiment 1 - Garbanzo beans

0 hr	1	a	19.2 ± 7.6	33.9	1.9	0.6	87.1
		b	92.4 ± 15.9				
24 hr	1	a	46.6 ± 7.2	36.8	0.8	0.6	93.6
		b	90.8 ± 15.0				
120 hr	1	a	103.7 ± 10.2	8.1	0.2	0.3	91.2
		b	60.6 ± 21.2				

Callosobruchus maculatus - Experiment 2 - Garbanzo beans

0 hr	1	a	45.4 ± 6.9	51.2	0.6	0.7	96.5
		b	103.4 ± 14.4				
24 hr	1	a	71.7 ± 7.5	33.7	0.03	0.6	99.7
		b	90.1 ± 15.5				
120 hr	1	a	129.6 ± 8.3	2.3	0.6	0.09	55.2
		b	25.8 ± 17.2				

Zabrotes subfasciatus - Experiment 1 - Blackeyed peas

0 hr	5	a	156.2 ± 14.6	41.7	0.2	0.6	98.2
		b	2.5 ± 0.5				
24 hr	1	a	88.0 ± 10.8	15.6	0.5	0.4	91.4
		b	88.8 ± 22.5				
120 hr	1	a	106.6 ± 7.5	9.4	0.6	0.3	83.9
		b	47.9 ± 15.7				

			Zabrotes subfasciatus - Experiment 2 - Blackeyed peas				
0 hr	6	a	51.1 ± 20.4	59.1	2.1	0.8	96.8
		b	-7.6 ± 5.6				
		c	129.3 ± 26.4				
24 hr	1	a	68.3 ± 5.7	101.4	1.5	0.8	96.1
		b	120.1 ± 11.9				
120 hr	1	a	136.7 ± 7.0	10.4	1.6	0.3	70.1
		b	47.3 ± 14.7				
			Zabrotes subfasciatus - Experiment 1 - White navy beans				
0 hr	1	a	18.0 ± 2.6	257.6	0.4	0.9	99.5
		b	88.4 ± 5.5				
24 hr	1	a	53.6 ± 3.8	48.4	0.6	0.7	96
		b	55.0 ± 7.9				
120 hr	1	a	87.6 ± 6.8	1.2	1.8	0.05	19.5
		b	15.6 ± 14.2				
			Zabrotes subfasciatus - Experiment 2 - White navy beans				
0 hr	5	a	110.1 ± 6.7	87.8	0.1	0.8	99.7
		b	2.9 ± 0.4				
24 hr	1	a	77.5 ± 4.3	37.2	0.7	0.6	94.1
		b	53.9 ± 8.8				
120 hr	1	a	90.7 ± 4.3	10.8	1.3	0.3	73.8
		b	29.7 ± 9.0				

[a]Regression equations used: 1) [asymptotic] $y=a+be^{-x}$; 2) [sigmoid] $y=a+b/(1+\exp(-(x-c)/d))$; 3) [exponential] $y=a+b\exp(-x/c)$; 4) [linear] $y=a+bx$; 5) [exponential] $y=a\exp(-x/b)$; 6) $y=a+bx+ce^{-x}$.

[b]Parameters for each regression equation.

[c]$F_{reg}=(SS_{Regression}/df_{Regression})\div(SS_{Residual}/df_{Residual})$. All values of F are significant at $P\leq 0.05$ with the exception of *C. maculatus* on garbanzo beans, Exp. #2, at 120 hr (F significant at $P \leq .25$; and *Z. subfasciatus* on white navy beans, Exp. #1, at 120 hr (F significant at $P \leq .5$).

[d]$F_L=(SS_{Lack\text{-}Of\text{-}Fit}/df_{Lack\text{-}Of\text{-}Fit})\div(SS_{Pure\ Error}/df_{Pure\ Error})$. None of the reported values of F indicate a signigicant lack of fit at $P \leq 0.05$.

[e]% of maximum r^2 possible $=[((SS_{Regression}-SS_{Total}+SS_{Corrected\ Total})/(SS_{Corrected\ Total})\div$

$((SS_{Corrected\ Total}-SS_{Pure\ Error})/SS_{Corrected\ Total})\times 100\%]$

Table 4. Analysis of variance results for experiments evaluating time of predator addition and predator density for each bruchid - legume combination.

Source	df	F	Pr>F
	Acanthoscelides obtectus - Blackeyed peas		
Xylocoris flavipes pairs (XF)	4, 120	32.9	<0.01
Time predators added (TIME)	2, 120	0.8	0.45
Experiment (EXP)	1, 120	17.2	<0.01
XF*EXP	4, 120	14.2	<0.01
XF*TIME	8, 120	0.7	0.72
TIME*EXP	2, 120	0.3	0.74
XF*TIME*EXP	8, 120	0.2	0.99
	Acanthoscelides obtectus - White navy beans		
Xylocoris flavipes pairs (XF)	4, 120	39.0	<0.01
Time predators added (TIME)	2, 120	0.5	0.60
Experiment performed in time (EXP)	1, 120	0.0	0.92
XF*EXP	4, 120	0.0	1.00
XF*TIME	8, 120	0.4	0.92
TIME*EXP	2, 120	2.1	0.12
XF*TIME*EXP	8, 120	2.0	0.06

	Callosobruchus analis - Blackeyed peas		
Xylocoris flavipes pairs (XF)	4, 120	241.0	<0.01
Time predators added (TIME)	2, 120	35.4	<0.01
Experiment performed in time (EXP)	1, 120	2.5	0.12
XF*EXP	4, 120	0.3	0.90
XF*TIME	8, 120	2.5	0.02
TIME*EXP	2, 120	5.3	<0.01
XF*TIME*EXP	8, 120	1.2	0.33
	Callosobruchus chinensis - Blackeyed peas		
Xylocoris flavipes pairs (XF)	4, 120	143.1	<0.01
Time predators added (TIME)	2, 120	10.7	<0.01
Experiment performed in time (EXP)	1, 120	12.5	<0.01
XF*EXP	4, 120	8.6	<0.01
XF*TIME	8, 120	1.8	0.10
TIME*EXP	2, 120	1.1	0.30
XF*TIME*EXP	8, 120	0.6	0.80
	Callosobruchus maculatus - Blackeyed peas		
Xylocoris flavipes pairs (XF)	4, 120	85.6	<0.01
Time predators added (TIME)	2, 120	145.6	<0.01

Experiment performed in time (EXP)	1, 120	219.0	<0.01
XF*EXP	4, 120	0.9	0.48
XF*TIME	8, 120	7.5	<0.01
TIME*EXP	2, 120	12.0	<0.01
XF*TIME*EXP	8, 120	0.9	0.51
	Callosobruchus maculatus - Garbanzo beans		
Xylocoris flavipes pairs (XF)	4, 120	30.0	<0.01
Time predators added (TIME)	2, 120	58.2	<0.01
Experiment performed in time (EXP)	1, 120	19.6	<0.01
XF*EXP	4, 120	0.2	0.96
XF*TIME	8, 120	2.0	0.06
TIME*EXP	2, 120	0.6	0.53
XF*TIME*EXP	8, 120	0.5	0.86
	Zabrotes subfasciatus - Blackeyed peas		
Xylocoris flavipes pairs (XF)	4, 120	49.4	<0.01
Time predators added (TIME)	2, 120	44.4	<0.01
Experiment performed in time (EXP)	1, 120	0.8	0.37
XF*EXP	4, 120	0.9	0.49
XF*TIME	8, 120	4.6	<0.01
TIME*EXP	2, 120	6.2	<0.01
XF*TIME*EXP	8, 120	1.1	0.37

Zabrotes subfasciatus - White navy beans

Xylocoris flavipes pairs (XF)	4, 120	51.5	<0.01
Time predators added (TIME)	2, 120	81.4	<0.01
Experiment performed in time (EXP)	1, 120	30.1	<0.01
XF*EXP	4, 120	0.7	0.57
XF*TIME	8, 120	6.1	<0.01
TIME*EXP	2, 120	2.7	0.07
XF*TIME*EXP	8, 120	1.2	0.30

Table 5. Analysis of variance results for experiments comparing *Xylocoris flavipes* strains.

Source	df	F	Pr>F
	Callosobruchus chinensis		
Xf strain (STRAIN)	2, 24	38.5	<0.01
Experiment replicate (EXP#)	1, 24	7.3	0.01
STRAIN * EXP#	2, 24	0.2	0.80
	Callosobruchus maculatus		
Xf strain (STRAIN)	2, 24	224.3	<0.01
Experiment replicate (EXP#)	1, 24	2.6	0.10
STRAIN * EXP#	2, 24	0.1	1.00
	Zabrotes subfasciatus		
Xf strain (STRAIN)	2, 24	52.7	<0.01
Experiment replicate (EXP#)	1, 24	5.2	0.03
STRAIN * EXP#	2, 24	1.7	0.20

Table 6. Analysis of variance and pairwise comparisons for each experiment with *X. flavipes* strains.

Bruchid	Experiment	F	Pr>F	Pair	Dunnett's MSD	Difference (95% CL)
C. chinensis	1	134.1	<0.01	XFR-C	9.6	59.8 (50.2, 69.4)
				XFS-C		68.8 (59.2, 78.4)
C. chinensis	2	10.5	<0.01	XFR-C	34.6	52.2 (17.6, 86.8)
				XFS-C		72.8 (38.2, 107.4)
C. maculatus	1	123.6	<0.01	XFR-C	29.7	177.4 (147.8, 207.1)
				XFS-C		203.0 (173.4, 232.7)
C. maculatus	2	100.7	<0.01	XFR-C	33.8	183.8 (150.0, 217.6)
				XFS-C		207.8 (174.0, 241.6)
Z. subfasciatus	1	41.7	<0.01	XFR-C	27.6	94.0 (66.3, 121.6)
				XFS-C		111.2 (83.6, 138.8)
Z. subfasciatus	2	15.8	<0.01	XFR-C	31.2	66.2 (35.0, 97.3)
				XFS-C		76.6 (45.4, 107.8)

Figure 3. Influence of predator density on number of F_1 bruchid progeny when predators were added 0 h (●), 24 h (■), or 120 h (◆) after the prey in Experiment 1 (A, C) and Experiment 2 (B, D). Time of predator introduction had no effect on *A. obtectus*, (E) on blackeyed peas - Experiment 1 (▲) and 2 (▼), or (F) on white navy beans - Experiment 1 and 2 combined (◆).

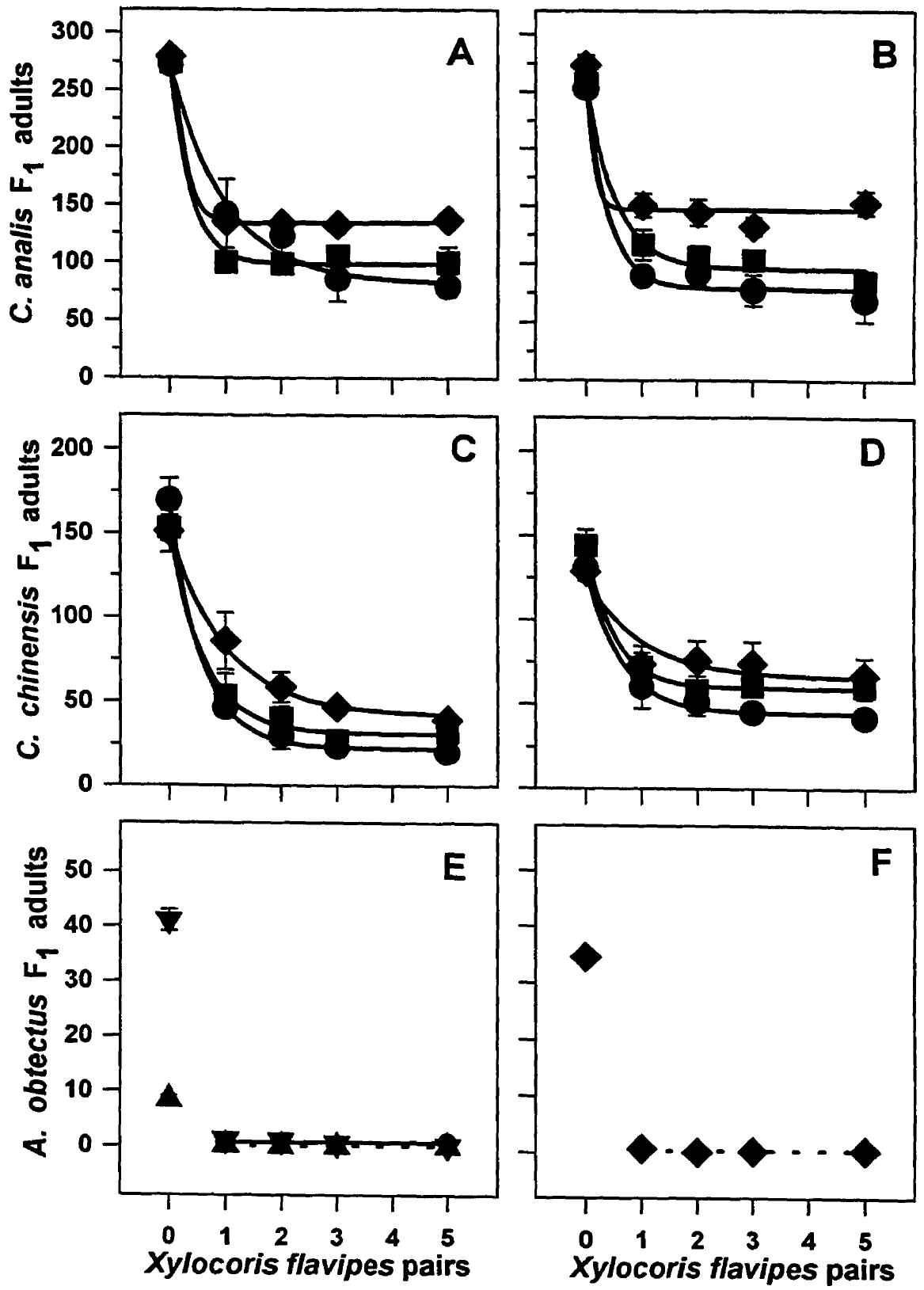

A
B
C
D
E
F
C. analis F1 adults
C. chinensis F1 adults
A. obtectus F1 adults
300
250
200
150
100
50
0
40
30
20
10
0 1 2 3 4 5
Xylocoris flavipes pairs
Xylocoris flavipes pairs

Figure 4. Influence of predator density on number of emerging F_1 *C. maculatus* progeny when predators were added 0 hr (●); 24 hr (■); or 120 hr (◆) after the prey. (A) and (B) Experiments 1 and 2, respectively, on blackeyed peas; (C) and (D) Experiments 1 and 2, respectively, on garbanzo beans.

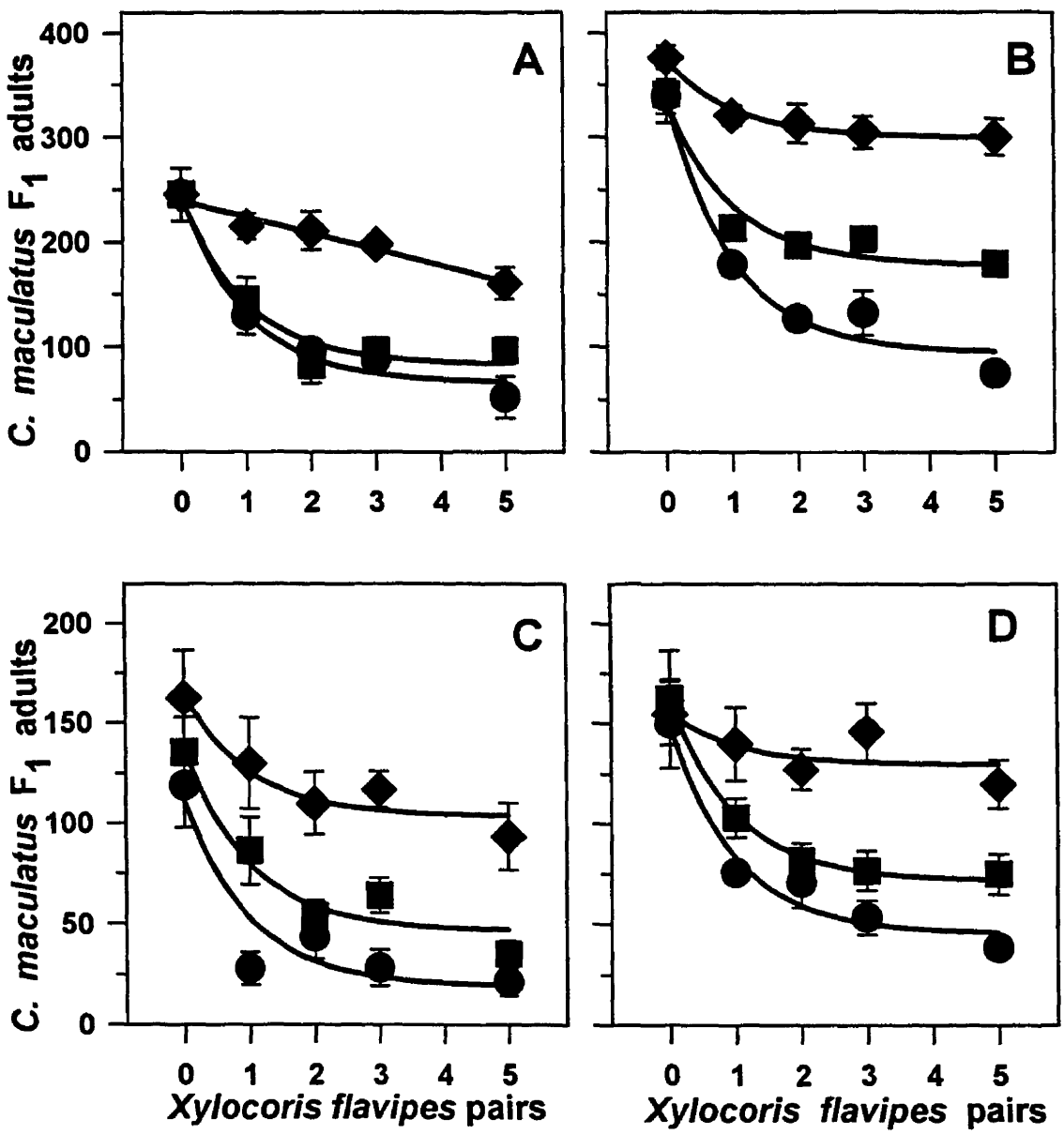

A
B
C
D
C. maculatus F1 adults
400
300
200
100
0
150
50
0 1 2 3 4 5
Xylocoris flavipes pairs

Figure 5. Influence of predator density on number of emerging F_1 *Z. subfasciatus* progeny when predators were added 0 hr (●); 24 hr (■); or 120 hr (◆) after the prey. (A) and (B) Experiments 1 and 2, respectively, on blackeyed peas; (D) and (E) Experiments 1 and 2, respectively, on white navy beans.

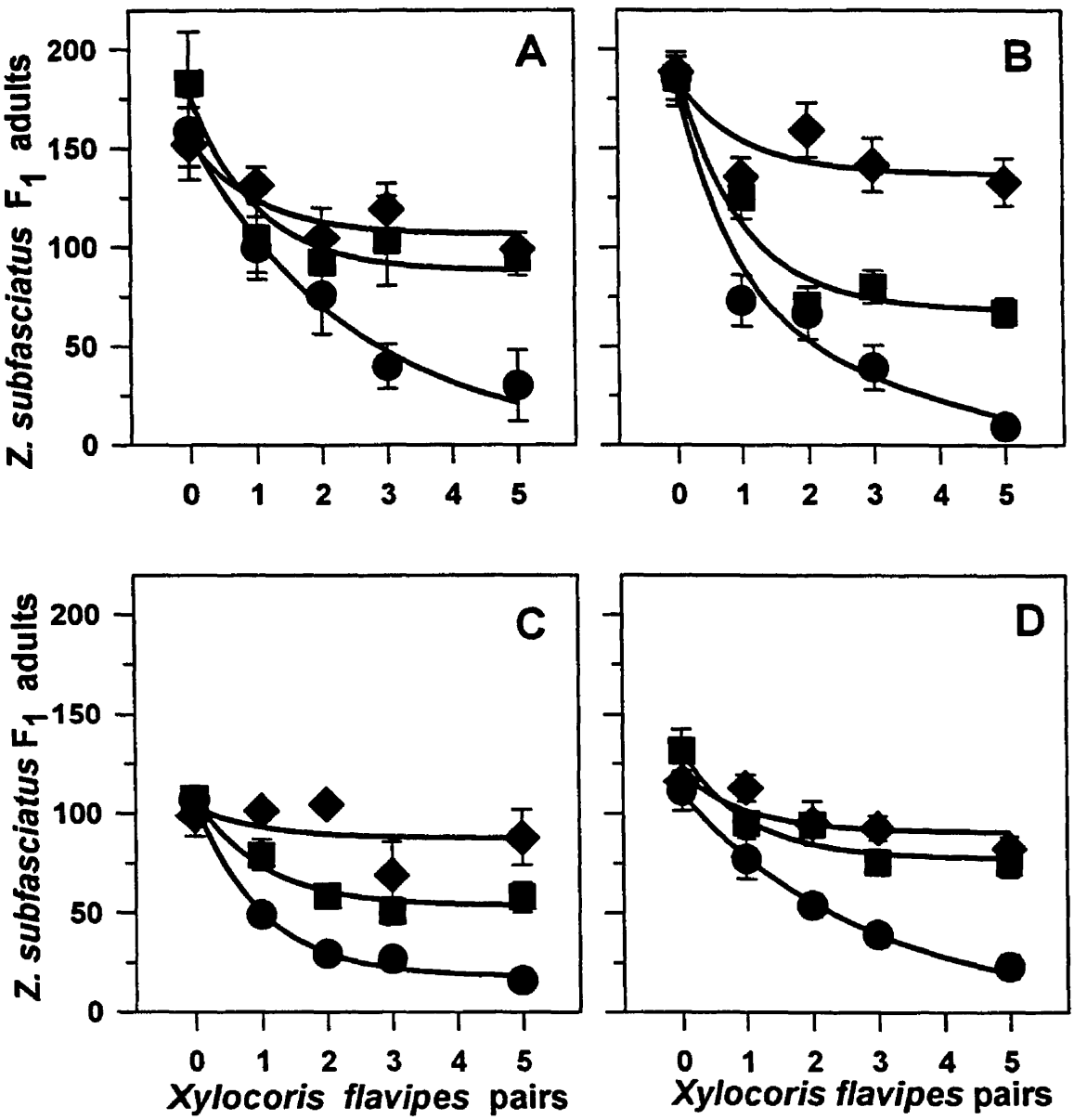
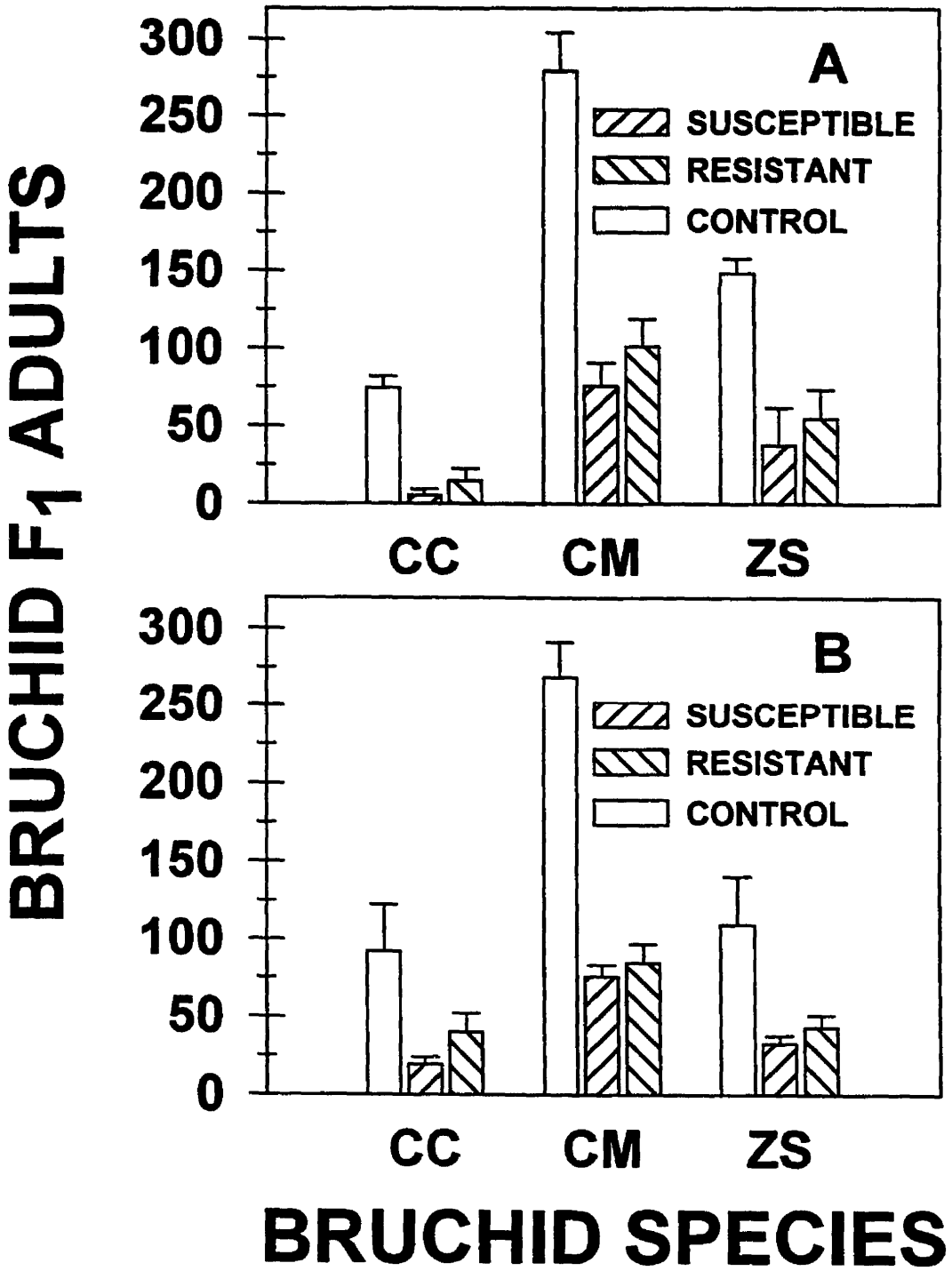

A
B
C
D
Z. subfasciatus F1 adults
Xylocoris flavipes pairs
0
50
100
150
200
0 1 2 3 4 5

Figure 6. Impact of malathion-resistant and malathion-susceptible strains of *Xylocoris flavipes* on numbers of emerging F_1 bruchid adults (CC - *Callosobruchus chinensis*, CM - *Callosobruchus maculatus*, ZS - *Zabrotes subfasciatus*, A - Experiment 1, B - Experiment 2).


BRUCHID F1 ADULTS
300
250
200
150
100
50
0
A
SUSCEPTIBLE
RESISTANT
CONTROL
CC
CM
ZS
300
250
200
150
100
50
0
B
SUSCEPTIBLE
RESISTANT
CONTROL
CC
CM
ZS
BRUCHID SPECIES

Connecting Statement III

Suppression of bruchid populations was influenced more by the timing of predator addition to experimental arenas than predator density. The optimal treatment occurred at the highest predator density (5 pairs) only when predators were added simultaneously with parental bruchids. Suppression was much higher than anticipated given the functional response of *X. flavipes* to adult bruchid prey and was probably the result of disrupted bruchid mating and oviposition. However, the inability of the predator to attack the internally-developing stages of most species examined limited the attainable level of biological control. Two larval parasitoids of numerous stored-product pests, *Anisopteromalus calandrae* and *Pteromalus cerealellae* were combined with the predator to determine if levels of suppression could be enhanced (Section IV).

Section IV: Predator and Parasitoid Combinations

Title

SUPPRESSION OF BRUCHIDS INFESTING STORED GRAIN LEGUMES WITH THE PREDATORY BUG *XYLOCORIS FLAVIPES* (REUTER)(HEMIPTERA: ANTHOCORIDAE): III. COMBINED PREDATOR/LARVAL PARASITOID TREATMENT

Abstract

Suppression of bruchid populations in blackeyed peas by the predatory bug *Xylocoris flavipes* was significantly enhanced by the addition of larval parasitoids. The parasitoid species evaluated, *Anisopteromalus calandrae* (Howard) and *Pteromalus cerealellae* (Ashmead) (Hymenoptera: Pteromalidae), are polyphagous on numerous stored-product pests, and have been reported in association with various bruchid species. Comparisons of the predator alone, various strains of each parasitoid species alone, and combinations of the predator with conditioned parasitoid species/strains showed significant suppression for all treatments. There was approximately 90% suppression with the natural enemy combinations. Intermediate suppression occurred with parasitoids alone, and the lowest levels of suppression, over 50%, occurred with *X. flavipes* alone. Parasitoid conditioning to host and commodity on biocontrol efficacy had little effect on either suppression of bruchids or parasitoid reproduction. There was no obvious manifestation of potential intraguild competition between the predatory bug and the larval parasitoids. Suppression achieved with a pesticide-resistant field strain of *A. calandrae* was slightly lower than with the susceptible laboratory strain. The results of this study indicate that a well-timed release of either parasitoid could significantly increase bruchid suppression when combined with *X. flavipes*.

Introduction

Anisopteromalus calandrae (Howard) and *Pteromalus calandrae* (Ashmead) (Hymenoptera: Pteromalidae) are polyphagous parasitoids of internally-developing coleopteran and lepidopteran stored-product pests (Brower et al, 1996). *A. calandrae* has been associated with the bruchid host species *Callosobruchus maculatus* (F.), *C. chinensis* (L.), and *Zabrotes subfasciatus* (Boheman) infesting various stored grain legumes (Ryoo and Chun, 1993; Heong, 1982; and Kistler, 1985). *P. cerealellae*, previously thought to be a monophagous parasitoid of *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelichiidae), is now known to parasitize *Callosobruchus maculatus* and other coleopteran stored-product pests (Brower, 1991). Laboratory tests evaluating the efficacy of the predatory bug *Xylocoris flavipes* (Reuter) (Hemiptera: Anthocoridae) in suppressing bruchid populations indicate that levels of bruchid progeny can be significantly reduced either directly by predation of parental bruchids or indirectly by the disruption of mating and oviposition (Sing, unpublished). Because protectively shielded eggs and internally-developing larvae and pupae are inaccessible prey for *X. flavipes* (Arbogast, 1978), experimentation was undertaken to determine if suppression levels could be enhanced by a combined treatment with the predator and a larval parasitoid. A similar approach to biological control was described by Howard and Fiske (1911) who used a sequence of parasitoid species to suppress specific, successive stages of lepidopteran pests. Compatability of natural enemies cannot be assumed; Press et al. (1974) reported that *X. flavipes* preyed upon the larvae of the parasitoid *Bracon hebator* Say in a test to evaluate their combined

and separate efficacy in controlling the Indianmeal moth, *Plodia interpunctella* (Hubner), clearly an example of intraguild predation (Rosenheim et al, 1995).

The purpose of the current study was to compare the ability of *A. calandrae* and *P. cerealellae* to suppress populations of *C. chinensis*, *C. maculatus*, and *Z. subfasciatus* infesting blackeyed peas, and to compare their reproduction on these host species. Previous experimentation indicated that parasitism by *P. cerealellae* is affected more by host suitability and various aspects of seed compatability than conditioning to a particular host or seed (Smith et al, 1995). Parasitoid strains evaluated in the current study included two field strains of *A. calandrae* collected from wheat infested with different hosts from two geographic regions (*Sitophilus oryzae*/South Carolina; *Sitophilus granarius*/Wisconsin) and a long-term lab strain reared on wheat infested with *Sitophilus oryzae* at the USDA-ARS Stored-Product Insects Research and Development Laboratory in Savannah, Georgia. Unconditioned parasitoids maintained on their original host and commodity were compared to parasitoids of the same strain reared on *C. chinensis*, *C. maculatus*, or *Z. subfasciatus* on blackeyed peas. These were compared to similarly conditioned and unconditioned laboratory strains of *P. cerealellae*, the unconditioned strain reared on *S. cerealella* infesting whole kernel wheat. The South Carolina strain of *A. calandrae* is known to be resistant to the organophosphates malathion, chlorpyrifos-methyl, and pirimiphos-methyl (Baker and Weaver, 1994), and the pyrethroids deltamethrin and cyfluthrin (Baker, 1994) while the Savannah or lab

strain is susceptible. Therefore, differing levels of bruchid and parasitoid progeny resulting from arenas treated with resistant or susceptible *A. calandrae* strains were evaluated as possible indicators of the fitness costs of resistance (Croft, 1990).

Materials and Methods

All hosts, predator strains, and parasitoid species/strains were reared at conditions of 29 ± 2°C, 65 ± 5% RH and a 12 h photophase:12 h scotophase. Experiments were performed under identical conditions. Conditioned parasitoids from the three strains of *A. calandrae* (Savannah - ACSAVC, South Carolina - ACSCC, and Wisconsin - ACWIC), and *P. cerealellae* (PCC) were routinely cultured according to the following protocol: 100 newly-emerged individuals were added to 0.95 ml glass Mason jars containing approximately 400 ml blackeyed peas infested 14 days previously with newly-emerged adult bruchids to provide third instar larval hosts. Unconditioned parasitoids were cultured on whole kernel wheat infested 21 days previously with newly emerged adults of the host species on which the parasitoid species or strain had originally been collected (unconditioned *A. calandrae* strains: ACSAVU, ACSCU, ACWIU; unconditioned *P. cerealella*: PCU). *X. flavipes*, maintained in continuous culture at the Savannah lab for more than 20 years, was reared in 3.78-liter glass jars fitted with Hexcell® paperboard harborage and fed frozen eggs of *Plodia interpunctella* (Hübner).

Experimental arenas consisted of 0.24-liter Mason jars filled with 100 g of equilibrated blackeyed peas, with five replicate jars per

treatment, five 0-48 hr old female and male bruchids sexed according to Halstead (1963) added to each arena. Five 0-7 d female and male predators, sexed according to Arbogast et al (1971), were added to each predator or predator/parasitoid treatment jar at the same time that the bruchids were added. Five newly emerged (0-48 h) male and female parasitoids were added to each parasitoid or predator/parasitoid treatment jar 14 d after the bruchids or bruchids/predators were added. The jars were held under experimental conditions for a total of 32 d (2n-10, n=approximate emergence time of one bruchid generation, or 21 d, minus 10 d to assure that only the F_1 generation was being counted, according to Howe and Currie, 1964), then frozen. The contents of each jar were sifted through a U.S. standard #6 sieve and the insect numbers recorded. There were fourteen natural enemy treatments for each bruchid species; all treatments were performed simultaneously though separate experiments were conducted for each bruchid species. The experiment was performed twice for each bruchid species. Non-parasitoid treatments included control and *X. flavipes*. Parasitoid treatments included the following for all strains/species of parasitoids: parasitoid conditioned to bruchid host and blackeyed peas; parasitoid unconditioned to bruchid host/reared on original host and commodity; conditioned parasitoid used in combination with lab strain *X. flavipes*.

The treatments available resulted in an unbalanced experiment overall. Therefore, within each bruchid species, the following were subjected to ANOVA (SAS Institute, 1988) to evaluate the effect of treatment or of replicate experiment on bruchid progeny numbers: 1) unconditioned lab strain natural enemies; 2) conditioned lab strain

parasitoids; 3) combinations of conditioned lab strain parasitoids with *X. flavipes*; and 4) combinations of conditioned *A. calandrae* field strains with *X. flavipes*. A significance level of $\alpha = 0.01$ was used. The controls suggested that because each experimental replicate used a new lot of beans, resulting variability in available host and prey numbers probably contributed more to variance than inconsistent natural enemy performance. For each of these individual treatments within a group (for both replicate experiments combined or for individual experiments if replicate experiment was significant in the ANOVA), treatment and control means were compared using Dunnett's one-tailed t-tests. ANOVA was also used to evaluate differences in parasitoid progeny between each of the groupings above, once again to discriminate contributions of either treatment or replicate experiment to total variation. Finally, ANOVA was also used to evaluate contributions of treatment and replicate experiment variability to the overall variability in the numbers of bruchid and parasitoid progeny within conditioned or unconditioned strains of the parasitoid *A. calandrae*.

Results

All natural enemy treatments had a significant effect on progeny abundance for each bruchid species (Figures 1 & 2, Tables 1-4). Suppression levels achieved by the treatments ranged from about 75% for *X. flavipes* (XF) alone against any bruchid species (Fig. 1 & 2) to greater than 90% for most predator/prey combinations (PP) (Fig. 1 & 2). The efficacy of the parasitoid species treatments (PC, PU) was generally greater than that of the predator alone (XF) (Table 1; Fig. 1 & 2). The

combined predator/conditioned parasitoid treatments (PP) had the most dramatic impact on bruchid reproductive success (Tables 3 & 4) for each bruchid species (Fig. 1 & 2), and the level of suppression was influenced by the parasitoid species used in combination with *X. flavipes* (Tables 3 & 4).

Overall enhancement of suppression by the combination of *X. flavipes* with a larval parasitoid was greatest against *C. maculatus*, the most fecund pest species evaluated in this study (Fig. 1 & 2). This was more evident for combinations including *P. cerealellae* than for those with *A. calandrae*, which gave differing results in Experiment 1 and 2 (Fig. 1 & 2, Table 3 & 4).

Parasitoid reproduction (Fig. 3 & 4) was quite consistent for all parasitoid treatments (Tables 1-4) with minor fluctuations due to replicate experiment, mirroring differences in host levels in untreated arenas (C) (Fig. 1 & 2). The added benefits of the combination treatments can best be assessed visually in Figures 3 and 4. In each of these, PCXF parasitoid progeny represents control of F_1 bruchid progeny that were not suppressed by the addition of *X. flavipes* simultaneously with the bruchids.

Unconditioned (PU) and conditioned (PC) parasitoid strains suppressed bruchid populations equally and reproduced with similar success (Table 1, 3, 5 & 6), but here again, parasitoid species was a significant factor with *A. calandrae* being more capable than *P. cerealellae* at both (Fig. 1 & 2). Also, the visual assessment of Figures 1 and 3 indicates that the suppression by and reproduction of field compared to laboratory strains of *A. calandrae* was very similar.

Since these strains also show differing levels of insecticide susceptibility, this physiological difference as well has a minimal impact on parasitism or reproduction (Fig. 1 & 3).

Discussion

Overall, every biocontrol treatment significantly suppressed each bruchid species relative to the control treatments. There were no obvious differences due to conditioning or strain within treatments for any bruchid species, probably due to the known polyphagous nature of the natural enemies evaluated here. Furthermore, these results indicate that neither the biocontrol efficacy nor the fecundity of the pesticide resistant strains were significantly compromised when compared to their susceptible counterparts. Additionally, field strains of *A. calandrae* collected from geographically and environmentally disparate regions demonstrated similar biocontrol potential against non-typical host pests. For the less fecund bruchid species, *C. chinensis* and *Z. subfasciatus*, all parasitoid species and strains performed equally well. The predator only treatment was less effective than any parasitoid only treatment against all three pest species. *P. cerealellae* was less effective than *A. calandrae* in suppressing the most fecund pest, *C. maculatus*. This finding is significant because it indicates that within this system, *P. cerealellae* can successfully parasitize fewer hosts than *A. calandrae*.

Data from the combination treatments in this study indicated that differing levels of pest suppression will result when natural enemies are used alone and concurrently. In general the combination treatments

were the most effective when the three treatments were compared. Most significant perhaps is the implication of differing parasitoid progeny levels found in the combination treatments compared to the parasitoid only treatments. Disturbance and predation occurring during bruchid oviposition in the combination treatments when (*X. flavipes* was added similtaneously to the treatment arena) with the bruchids resulted in a lower number of hosts for the parasitoids to utilize and consequently reduced their number of progeny. For treatments in which there was little difference in bruchid progeny level between in the parasitoid alone and predator/parasitoid combination treatment, fewer parasitoid progeny in the combination treatment indicates that because there were fewer internally developing hosts, there would be less overall damage to the commodity.

The practical implications of this research are that high levels of suppression are possible with precisely-timed releases of parasitoids following predator introduction at the initial time of bean storage. However, the ideal timing of parasitoid releases under field conditions is subject to a number of environmental variables influencing host stage phenology within on-going field infestation.

Biological control depends upon the fitness of a proposed control agent to both survive in a specific pest's environment and to effectively control (predate or parasitize) the pest population (Messenger et al. 1976). In this context, fitness is delimited by the degree of host and/or habitat specificity. However, fitness may theoretically be superseded by the importance of first bringing the biocontrol agent and its potential host together. Many host-specific

natural enemies are aided in the essential task of host foraging (Lewis et al. 1990) or location of a potential host community (Vinson, 1984) by complex semiochemical cues usually transmitted from an amalgamation of host and host's host plant products. The combined plasticity and efficacy of the biological control agents observed in the following study indicates that the success of habitat-specific natural enemies arising in established colonies is perhaps more influenced by the unique opportunities and properties endemic to that environment than to specific host species or commodities (the equivalent of the host's host plant).

In conclusion, the development of sequentially administered biological control merits futher investigation in applications where predictive modelling can anticipate the circumstances under which suitable stage-specific natural enemies can be used in effective non-competitive combinations.

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Table 7. Analysis of variance for contribution of unconditioned natural enemy treatment and replicate experiment to variability in numbers of bruchid and parasitoid progeny for each bruchid species and pair-wise comparisons for each treatment with the control.

Unconditioned lab strain natural enemy treatments			
Callosobruchus chinensis			
bruchid progeny			
Source	df	F	Pr>F
natural enemy treatment	3, 32	86.72	<0.001
experiment	1, 32	3.58	0.068
pair-wise comparisons	Dunnett's MSD	difference	95% c. l.
ACSAVU-C	12.56	80.90	68.34 - 93.46
PCU-C		78.60	66.04 - 91.16
XF-C		70.80	58.24 - 83.36
parasitoid progeny			
Source	df	F	Pr>F
parasitoid species	1, 32	0.27	0.613
experiment	1, 32	0.34	0.568
Callosobruchus maculatus			
bruchid progeny			
Source	df	F	Pr>F
natural enemy treatment	3, 32	224.83	<0.001
experiment	1, 32	0.01	0.941
pair-wise comparisons	Dunnett's MSD	difference	95% c. l.
ACSAVU-C	22.40	251.40	229.00 - 273.80
PCU-C		189.70	167.30 - 212.10
XF-C		205.40	183.00 - 227.80

parasitoid progeny

Source	df	F	Pr>F
parasitoid species	1, 32	6.48	0.022
experiment	1, 32	6.35	0.023

Zabrotes subfasciatus

bruchid progeny

Source	df	F	Pr>F
natural enemy treatment	3, 32	131.07	<0.001
experiment	1, 32	4.38	0.044
pair-wise comparisons	Dunnett's MSD	difference	95% c. l.
ACSAVU-C	15.92	127.10	111.18 - 143.02
PCU-C		127.40	111.48 - 143.32
XF-C		93.90	77.98 - 109.82

parasitoid progeny

Source	df	F	Pr>F
parasitoid species	1, 32	1.51	0.237
experiment	1, 32	6.99	0.018

Table 8. Analysis of variance for contribution of conditioned laboratory strain parasitoid treatment and replicate experiment to variability in numbers of bruchid and parasitoid progeny for each bruchid species and pair-wise comparisons for each treatment with the control.

Conditioned lab strain parasitoid treatments			
Callosobruchus chinensis			
bruchid progeny			
Source	df	F	Pr>F
parasitoid species	2, 24	102.70	<0.001
experiment	1, 24	1.91	0.179
pair-wise comparisons	Dunnett's MSD	difference	95% c. l.
ACSAVC-C	12.92	80.40	67.48 - 93.32
PCC-C		79.30	66.38 - 92.22
parasitoid progeny			
Source	df	F	Pr>F
parasitoid species	1, 16	0.98	0.338
experiment	1, 16	22.91	<0.001
Callosobruchus maculatus			
bruchid progeny			
Source	df	F	Pr>F
parasitoid species	2, 24	347.29	<0.001
experiment	1, 24	0.55	0.465
pair-wise comparisons	Dunnett's MSD	difference	95% c. l.
ACSAVC-C	20.53	246.10	225.57 - 266.63
PCC-C		218.10	197.57 - 238.63
parasitoid progeny			
Source	df	F	Pr>F
parasitoid species	1, 16	5.78	0.029

experiment	1, 16	1.37	0.259

Zabrotes subfasciatus

bruchid progeny

Source	df	F	Pr>F
parasitoid species	2, 24	242.02	<0.001
experiment	1, 24	5.59	0.026

pair-wise comparisons	Dunnett's MSD	difference	95% c. l.
ACSAVC-C	13.45	127.40	113.95 - 140.85
PCC-C		127.80	114.35 - 141.25

parasitoid progeny

Source	df	F	Pr>F
parasitoid species	1, 16	10.02	0.006
experiment	1, 16	11.19	0.004

Table 9. Analysis of variance for contribution of combined conditioned laboratory strain parasitoids with *X. flavipes* treatment and replicate experiment to variability in numbers of bruchid and parasitoid progeny for each bruchid species and pair-wise comparisons for each treatment with the control.

Combination of conditioned lab strain parasitoids with *X. flavipes*			
Callosobruchus chinensis			
bruchid progeny			
Source	df	F	Pr>F
parasitoid species/XF	2, 24	110.42	<0.001
experiment	1, 24	1.57	0.223
pair-wise comparisons	Dunnett's MSD	difference	95% c. l.
ACSAVC/XF-C	12.84	82.70	69.86 - 95.54
PCC/XF-C		81.90	69.06 - 94.74
parasitoid progeny			
Source	df	F	Pr>F
parasitoid species/XF	1, 16	0.08	0.787
experiment	1, 16	7.00	0.018
Callosobruchus maculatus			
bruchid progeny			
Source	df	F	Pr>F
parasitoid species/XF	2, 24	541.98	<0.001
experiment	1, 24	3.25	0.084
pair-wise comparisons	Dunnett's MSD	difference	95% c. l.
ACSAVC/XF-C	18.29	259.40	241.11 - 277.69
PCC/XF-C		260.00	241.71 - 278.29
parasitoid progeny			
Source	df	F	Pr>F
parasitoid species/XF	1, 16	1.76	0.204
experiment	1, 16	0.00	0.985

Zabrotes subfasciatus

bruchid progeny

Source	df	F	Pr>F
parasitoid species/XF	2, 24	245.34	<0.001
experiment	1, 24	6.07	0.021
pair-wise comparisons	Dunnett's MSD	difference	95% c. l.
ACSAVC/XF-C	13.44	128.50	115.06 - 141.94
PCC/XF-C		128.30	114.86 - 141.74

parasitoid progeny

Source	df	F	Pr>F
parasitoid species/XF	1, 16	0.03	0.863
experiment	1, 16	5.32	0.035

Table 10. Analysis of variance for contribution of combined conditioned *A. calandrae* field strain parasitoids with *X. flavipes* treatment and replicate experiment to variability in numbers of bruchid and parasitoid progeny for each bruchid species and pair-wise comparisons for each treatment with the control.

Combination of conditioned *A. calandrae* field strains with *X. flavipes*			
Callosobruchus chinensis			
bruchid progeny			
Source	df	F	Pr>F
ACFSC/XF	2, 24	110.01	<0.001
experiment	1, 24	1.41	0.246
pair-wise comparisons	Dunnett's MSD	difference	95% c. l.
ACSCC/XF-C	12.83	82.40	69.57 - 95.23
ACWIC/XF-C		82.50	69.67 - 95.33
parasitoid progeny			
Source	df	F	Pr>F
ACFSC/XF	1, 16	0.05	0.819
experiment	1, 16	20.74	<0.001
Callosobruchus maculatus			
bruchid progeny			
Source	df	F	Pr>F
ACFSC/XF	2, 24	344.59	<0.001
experiment	1, 24	8.45	0.008
pair-wise comparisons	Dunnett's MSD	difference	95% c. l.
experiment 1:			
ACSCC/XF-C	42.32	240.80	198.49 - 283.11
ACWIC/XF-C		240.60	198.29 - 282.81
experiment 2:			
ACSCC/XF-C	20.21	267.40	247.20 - 287.61
ACWIC/XF-C		262.60	242.40 - 282.81

parasitoid progeny

Source	df	F	Pr>F
ACFSC/XF	1, 16	0.49	0.496
experiment	1, 16	10.67	0.005

Zabrotes subfasciatus

bruchid progeny

Source	df	F	Pr>F
ACFSC/XF	2, 24	247.19	<0.001
experiment	1, 24	5.44	0.028

pair-wise comparisons	Dunnett's MSD	difference	95% c. l.
ACSCC/XF-C	13.43	128.70	115.27 - 142.13
ACWIC/XF-C		128.80	115.37 - 142.23

parasitoid progeny

Source	df	F	Pr>F
ACFSC/XF	1, 16	0.78	0.389
experiment	1, 16	1.33	0.265

Table 11. Analysis of variance for contribution of conditioned *A. calandrae* strain treatment and replicate experiment to variability in numbers of bruchid and parasitoid progeny for each bruchid species.

Source	df	F	Pr>F
	Callosobruchus chinensis		
	bruchid progeny		
ACC	2, 24	0.97	0.394
experiment	1, 24	1.85	0.186
	parasitoid progeny		
ACC	2, 24	2.52	0.102
experiment	1, 24	3.44	0.076
	Callosobruchus maculatus		
	bruchid progeny		
ACC	2, 24	0.55	0.583
experiment	1, 24	28.39	<0.001
	parasitoid progeny		
ACC	2, 24	4.47	0.022
experiment	1, 24	0.35	0.559
	Zabrotes subfasciatus		
	bruchid progeny		
ACC	2, 24	1.64	0.215
experiment	1, 24	0.21	0.649
	parasitoid progeny		
ACC	2, 24	4.34	0.025
experiment	1, 24	2.34	0.139

Table 12. Analysis of variance for contribution of unconditioned *A. calandrae* strain treatment and replicate experiment to variability in numbers of bruchid and parasitoid progeny for each bruchid species.

Source	df	F	Pr>F
	Callosobruchus chinensis		
	bruchid progeny		
ACU	2, 24	0.57	0.575
experiment	1, 24	0.27	0.607
	parasitoid progeny		
ACU	2, 24	2.54	0.100
experiment	1, 24	1.52	0.230
	Callosobruchus maculatus		
	bruchid progeny		
ACU	2, 24	0.32	0.730
experiment	1, 24	27.36	<0.001
	parasitoid progeny		
ACU	2, 24	1.98	0.161
experiment	1, 24	10.42	0.004
	Zabrotes subfasciatus		
	bruchid progeny		
ACU	2, 24	1.46	0.252
experiment	1, 24	1.31	0.264
	parasitoid progeny		
ACU	2, 24	1.74	0.197
experiment	1, 24	2.88	0.103

Figure 7. Mean number of emerging F_1 bruchids for *A. calandrae* strains. Strains are: SAV= pesticide-susceptible, long-term laboratory strain; SC= pesticide-tolerant strain field-collected in South Carolina; WI= strain field-collected in Wisconsin. Natural enemy treatments are: C= control; XF= *X. flavipes* predator; PC= parasitoid conditioned to bruchid host and blackeyed peas; PU= parasitoid reared on original host and commodity; PP= predator combined with conditioned parasitoid. Empty bars represent Experiment 1 and filled bars represent Experiment 2. Error bars denote standard error.

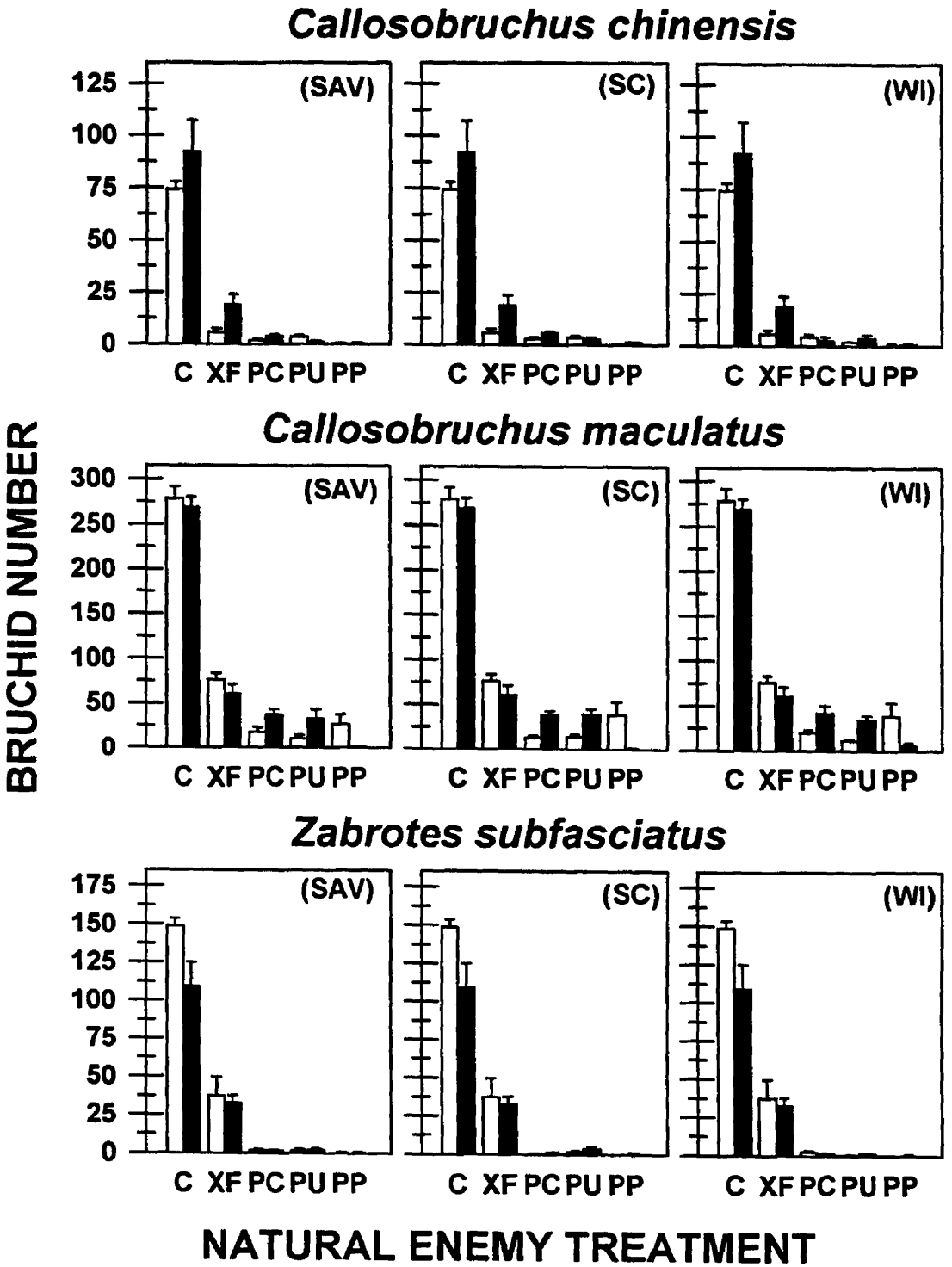

Callosobruchus chinensis
(SAV)
(SC)
(WI)
125
100
75
50
25
0
C XF PC PU PP
Callosobruchus maculatus
300
250
200
150
100
50
0
Zabrotes subfasciatus
175
150
125
100
75
50
25
BRUCHID NUMBER
NATURAL ENEMY TREATMENT

Figure 8. Mean number of emerging F_1 bruchids for *P. cerealellae* strains. Natural enemy treatments are: C= control; XF= *X. flavipes* predator; PC= parasitoid conditioned to bruchid host and blackeyed peas; PU= parasitoid reared on original host and commodity; PP= predator combined with conditioned parasitoid. Empty bars represent Experiment 1 and filled bars represent Experiment 2. Error bars denote standard error.

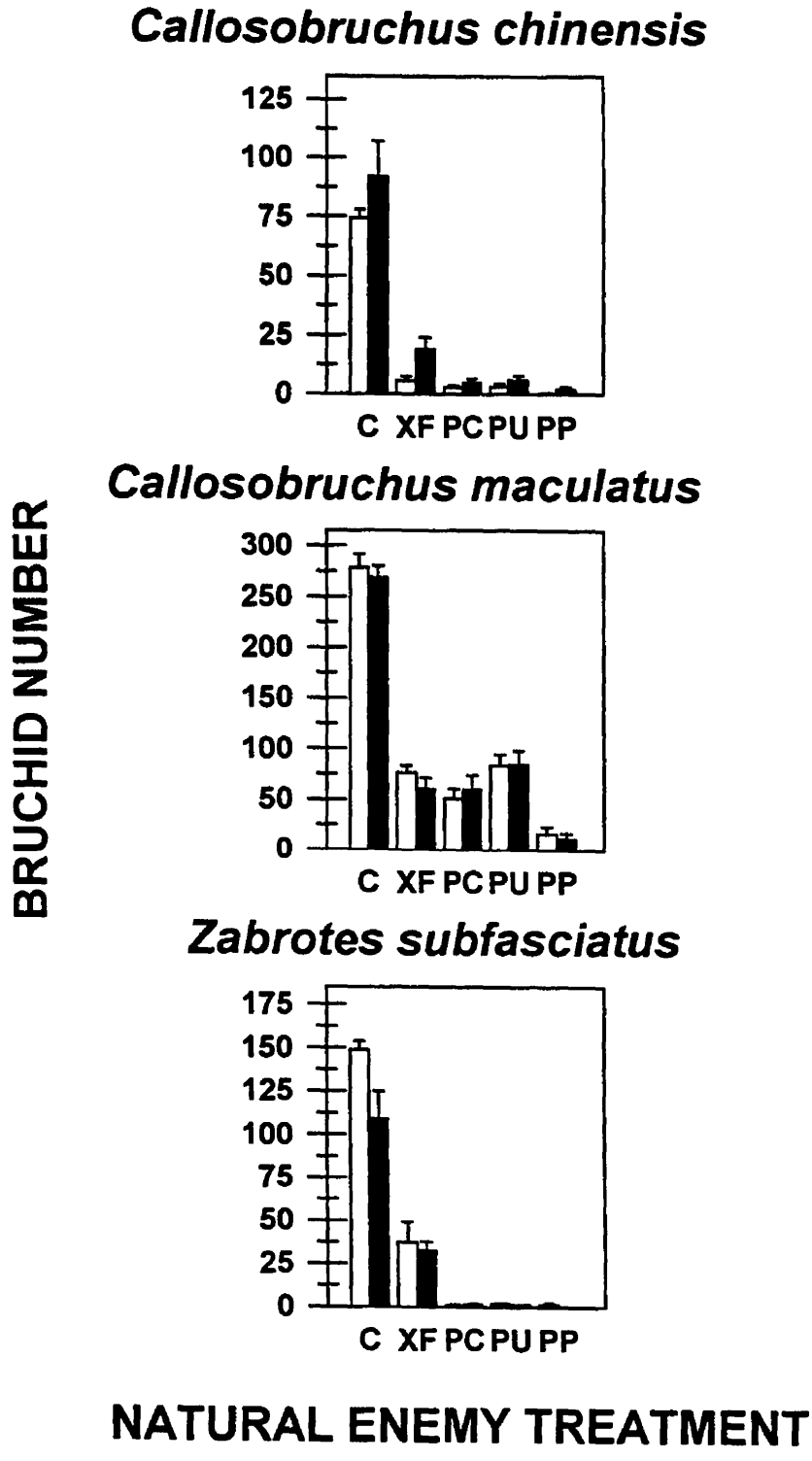

Callosobruchus chinensis
125
100
75
50
25
0
C XF PC PU PP
Callosobruchus maculatus
300
250
200
150
100
50
0
C XF PC PU PP
Zabrotes subfasciatus
175
150
125
100
75
50
25
0
C XF PC PU PP
BRUCHID NUMBER
NATURAL ENEMY TREATMENT

Figure 9. Mean number of emerging F_1 parasitoids in *A. calandrae* treatments. Strains are: SAV= pesticide-susceptible, long-term laboratory strain; SC= pesticide-tolerant strain field-collected in South Carolina; WI= strain field-collected in Wisconsin. Natural enemy treatments are: XF= *X. flavipes* predator; PC= parasitoid conditioned to bruchid host and blackeyed peas; PUC= parasitoid unconditioned, reared on original host and commodity; PCXF= predator combined with conditioned parasitoid. Empty bars represent Experiment 1 and filled bars represent Experiment 2. Error bars denote standard error.

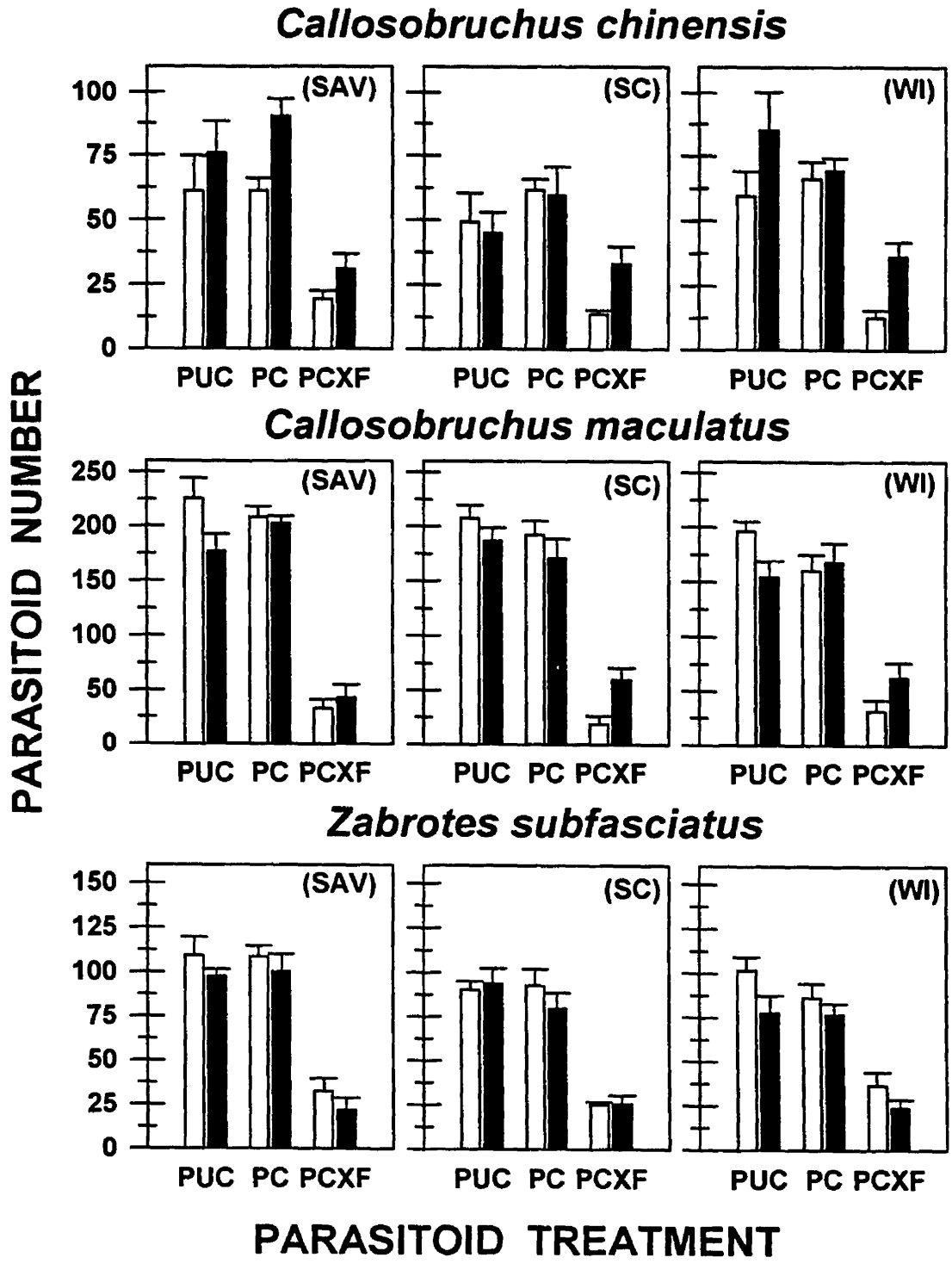
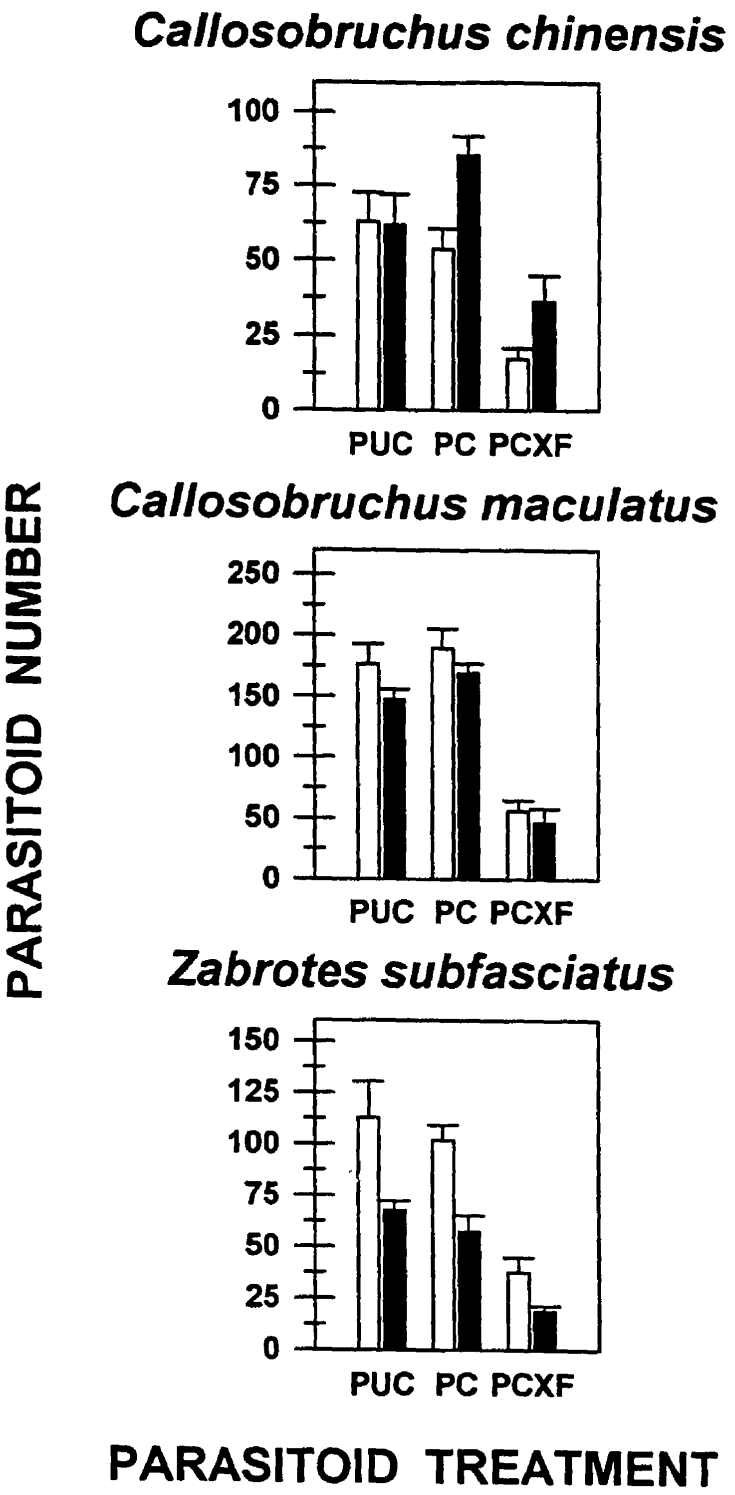

Callosobruchus chinensis
(SAV)
(SC)
(WI)
100
75
50
25
0
PUC PC PCXF
Callosobruchus maculatus
250
200
150
100
50
0
Zabrotes subfasciatus
150
125
PARASITOID NUMBER
PARASITOID TREATMENT

Figure 10. Mean number of emerging F_1 parasitoids for *P. cerealellae* treatments. Natural enemy treatments are: XF= *X. flavipes* predator; PC= parasitoid conditioned to bruchid host and blackeyed peas; PUC= parasitoid unconditioned, reared on original host and commodity; PCXF= predator combined with conditioned parasitoid. Empty bars represent Experiment 1 and filled bars represent Experiment 2. Error bars denote standard error.


Callosobruchus chinensis
100
75
50
25
0
PUC PC PCXF
Callosobruchus maculatus
250
200
150
100
50
0
PUC PC PCXF
Zabrotes subfasciatus
150
125
100
75
50
25
0
PUC PC PCXF
PARASITOID NUMBER
PARASITOID TREATMENT

General Conclusions

The functional response of Xylocoris flavipes to adult and immature bruchid prey. *X. flavipes* successfully subdued and killed adult bruchid prey at a low but consistent rate in spite of the size disparity between predator and prey. The female predator, larger in body size than its male counterpart, had a higher functional response to adult bruchid prey; there was also a negative correlation between mean prey body weight and rate of predation. Allomones unique to hemipteran predatory biology in the form of a directed scent-gland spray and injected digestive salivary venom probably play an essential role in *X. flavipe's* successful predation of large prey. Previous experimentation indicates that the predator will persistently attack `unpreferred' prey when more accessible prey is unavailable, a quality which increases *X. flavipe's* value as a generalist predator because it will attempt to feed on most available species, including any field pests that may be inadvertantly brought into the storage facility at harvest. Additionally, this trait, possibly an adaptation to counteract the extinction of local populations through starvation, also promotes the establishment of long-term predator populations in storage facilities. The discovery that *X. flavipes* can kill adult bruchids broadens the potential applications of a natural enemy already recognized as a key component in the biological control of diverse stored-product pests. The identification of *X. flavipes* as a predator of adult bruchids increases the number of known natural enemies of these pests and could contribute to the development of a viable pest management alternative where existing control methods are inaccessible or unreliable.

The biological control of many bruchid species is complicated by the inaccessibility of their immature stages: the eggs are cemented onto the bean with an impenetrable protective coating and neonate larvae hatch and immediately tunnel directly into the bean, where development continues in that secure and stable environment until adult emergence. Of the species discussed in this study, the only immature stages that can be attacked by *X. flavipes* are the eggs and early instar larvae of *A. obtectus*. The rate of predation on the larvae was particularly high, and possibly indicative of the predator's attraction to or preference for active prey. Because it

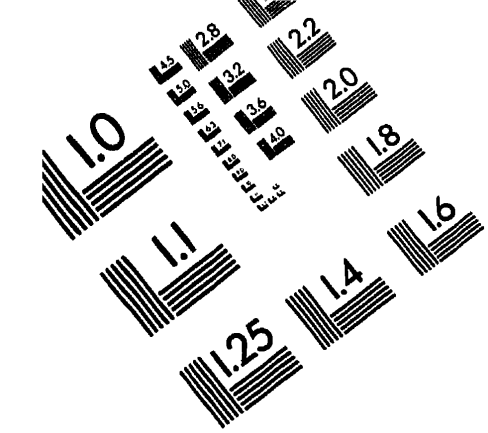
can attack both the adult and immature stages of *A. obtectus*, *X. flavipes* shows great potential for controlling this species and therefore further evaluation of its performance under field conditions should be investigated.

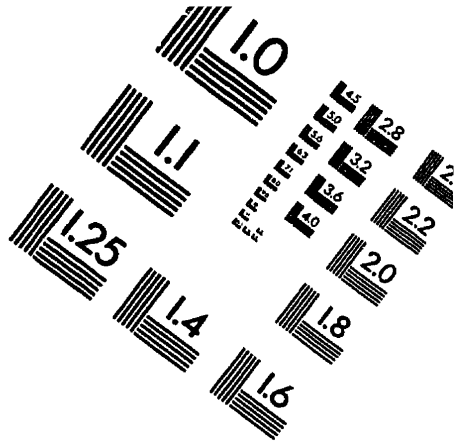
Population interactions between *X. flavipes* and various bruchid species. *X. flavipes* was capable of effectively searching out and preying upon adult bruchids infesting various grain legumes. Comparisons were made of the effects of predator density and time elapsed between bean infestation and predator addition on the number of emerging adult bruchid progeny. Results indicated that reduction of reproduction surpassed 50% for all bruchid species evaluated when the highest density of predators (5 pairs) were added simultaneously with the prey. Delaying predator introduction to arenas for even 24 hr significantly increased the level of bruchid progeny. Predator density contributed much less to treatment efficacy; the presence of a single mated pair of predators in an arena significantly reduced the number of bruchid progeny from that found in control arenas and was improved upon only when predator density jumped to five pairs per arena. Because the predator's functional response to adults was fairly low, it could be conjectured that the population suppression observed here was probably due more a function of mating and oviposition disruption than actual predation of the parental adults. A field-collected, pesticide-resistant strains of *X. flavipes* was slightly less effective than the pesticide-susceptible laboratory strain.

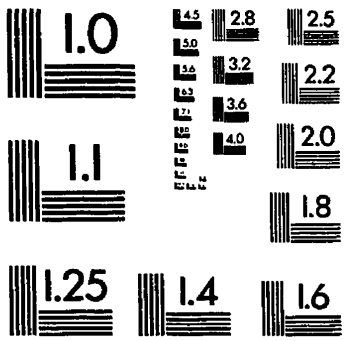
Combined predator/larval parasitoid treatments. Suppression of bruchid populations was significantly enhanced by pairing *X. flavipes* with either *A. calandrae* or *P. cerealellae*. Both of these pteromalid parasitoids have been evaluated and recognized as efficacious, polyphagous natural enemies of the later larval stages of numerous stored-product pests, including some Bruchidae. In this study, combining beneficial species that attack different specific developmental stages of the pest avoided the incidence of intraguild competition. Unlike parasitoids in other agricultural ecosystems, conditioning to prey or commodity did not significantly impact levels of pest suppression, nor did geographical, host, or commodity origin affect parasitoid performance. A field-collected, pesticide-resistant strain of *A. calandrae* was slightly less

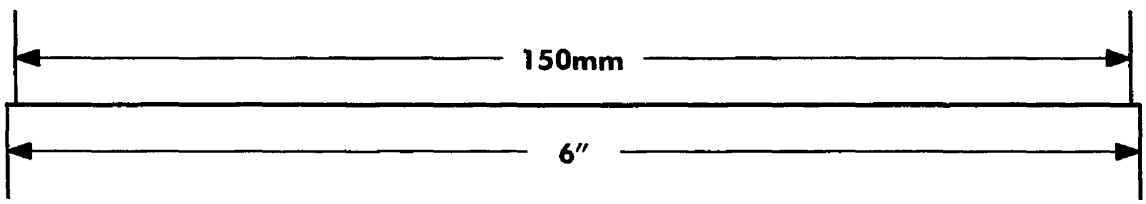
effective than the pesticide-susceptible laboratory strain.

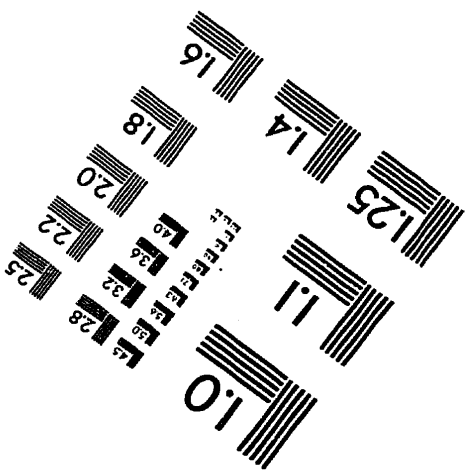
Possible areas for future investigation. Suppression of bruchid populations resulting from the combination of *X. flavipes* with larval parasitoids suggests that the addition of a third stage-specific natural enemy to the combined treatment, an egg parasitoid of the genus *Uscana*, may result in pest eradication, especially in the less fecund pest species. This approach could also circumvent the precise timing required to guage synchrony with prey/hosts for treatment with a single species of natural enemy, and could be applied at any time during storage. The development of effective biological control of bruchids is desirable not only because current methods inadequately address all permutations of the problem, but also because of the increasing global demand for high quality, low-cost alternatives to animal source dietary protein.

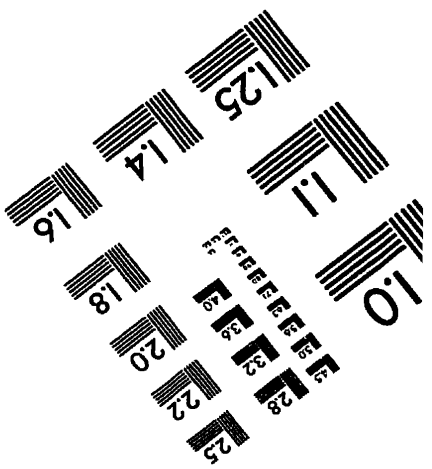












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