Decrease in population size of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) at two temperatures in different wheat cultivars by the parasitoid *Theocolax elegans* (Hymenoptera: Pteromalidae) *

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Abstract

A laboratory study was conducted to examine the role of the parasitoid *Theocolax elegans* in decreasing quantity of F1 *Rhyzopertha dominica* progeny. Experiments were conducted at two temperatures, 27.0 and 34.0℃, and in five divergent cultivars of U. S wheat. Wheat stored in glass jars was infested with *R. dominica* for four days and then the parental generation was removed while the progeny were allowed to complete their life cycle *Theocolax elegans* were released into half of the jars and their progeny were allowed to develop. Adult progeny of pest and parasitoid were collected and analyzed to access efficacy of the parasitoid at suppressing its host.

Increased suppression of the pest was detected at the lower temperature when compared with the upper temperature. More parasitoids completed their life cycle at the lower temperature, thus increasing the potential for control in future generations. Analysis of interactions among the three trophic levels indicated that wheat cultivars did not significantly enhance the reproductive capacity of the parasitoid.

Introduction

Biological control is an important part of integrated pest management (IPM) methods for stored grain. Parasitoids and predators occur naturally in bulk stored grain facilities (Nilakhe and Parker 1990, Vela de Garza 1993, Gates 1995) and are known to attack most insect pests of stored grain (Hagstrum and Flinn 1992). In 1992 the U. S Food and Drug Administration (Anonymous 1992) exempted beneficial insects from tolerance standards in stored grain. This has increased interest in using entomophagous insects, such as the parasitoid *Theocolax elegans* (Westwood) (Hymenoptera: Pteromalidae), in managing pests such as *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). *Rhyzopertha dominica* is one of the most damaging and abundant pests of stored grain in the southern United States (Cuperus et al. 1986, Vela-Coiffier et al. 1997). This insect feeds in grain both as a larva and as an adult. Detection of the species is difficult because larvae and adults tend to remain hidden inside the kernel (Fargo et al. 1989, Vela-Coiffier et al. 1997). Females feed extensively and then lay eggs singly or in small clusters exterior to the kernels (Golebiowska 1969). The first instar then bores into the kernel where it continues feeding on the endosperm, molting 3－5 times before pupation The complete life cycle takes approximately 25 days at 34.0℃ (Birch and Snowball 1945, Howe 1950) Optimal developmental temperatures for this species are 32.0 to 35.0℃ (White 1995) but development can be slowed by cooling the temperature of the grain (Howe 1950)

Theocolax elegans is a solitary ectoparasitoid that parasitizes beetle larvae within the kernel Sharifi (1972) provided an extensive list of possible hosts of *T elegans*. Potential sites for oviposition are determined through mechanical detection using the antennae (Van Den Assem and Kuenen 1958) During oviposition, the female inserts an egg into the kernel placing it on the exterior body surface of the host larva. The larva of the parasitoid continues to develop exterior to the pest larva while both are still confined within the kernel. *Theocolax elegans* can complete one generation in approximately 25 days at 25.0℃ (Van Den Assem and Kuenen 1958). The developmental time for *T. elegans* is approximately one-half that of its hosts (Flinn et al. 1996). After emergence, the adult parasitoid bores out of the kernel.

Few studies have been conducted utilizing augmentation of *T elegans* to suppress *R. dominica* populations. Flinn et al (1996) conducted experiments in steel grain bins in which this parasitoid species was able to suppress *R dominica* populations in excess of 90% for two years compared to the unparasitized group In addition, *R*

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dominica populations were suppressed well below the Federal Grain Inspection Service (FGIS) standard of two live insects per kg for the 'sample' grade designation. Flinn (1998) also demonstrated that augmentative releases of *T elegans* were more efficacious when coupled with lower temperatures

Ecologists use the term trophic or feeding level to delineate an organism's place in the food chain (Lindeman 1942) The management system of concern in this paper includes the wheat cultivar as the first trophic level, *R dominica* as the second, and finally *T elegans* as the third trophic level. Interactions among these three trophic levels are collectively termed 'tritrophic interactions' An important consideration among tritrophic interactions is that members of the first and third trophic levels may act in a mutualistic manner (Price 1986) Starks et al. (1972) studied relationships in a cereal field crop ecosystem and quantified important observations pertinent to other ecosystems Of primary concern in their study and several others was the concept that the primary trophic level, i e. the host plant of the herbivore, could influence development and survivorship of a carnivorous species at the third trophic level (Starks et al. 1972, Reed et al 1991, 1992, 1993, Reitz and Trumble 1996). A second important aspect of these studies was that activity of parasitoids at the third trophic level can influence the extent of feeding damage to the plant species Finally, biological control and host plant resistance can be highly compatible in the development of successful pest management programs (Salto et al. 1982).

Objectives of this study were to (1) evaluate the effects of wheat cultivar, temperature, and the parasitoid *T elegans*, on the number of adult *R dominica* progeny, (2) determine percent suppression of adult *R. dominica* progeny due to *T elegans*, (3) compare the ability of *T. elegans* to complete its life cycle under the influence of the different temperatures.

Materials and Methods

Five cultivars of wheat (*Triticum aestivum* L.) were procured from commercial seed producers and various foundation seed stocks across the United States. Wheat cultivars represented divergent genetic lineage and resistance to common field pathogens and insects. Selections included the hard red winter cultivar '2163' (Oklahoma Foundation Seed Stocks, Inc , Stillwater), soft red wheats 'Coker 916' (Novartis Seeds, Inc., Bay, Arkansas) and 'Wakefield' (Arkansas Agricultural Experiment Station, Fayetteville), the durum wheat 'Munich' (North Dakota Seedstocks Project, Fargo) and a white spring wheat, 'Wawawai' (Washington State Crop Improvement Association, Pullman) Following receipt wheat samples were frozen for one week to eliminate the possibility of existing infestations Grain was then cleaned by multiple passages over a seed cleaner (Clipper model M2B, Bluffton Agricultural Industrial Corp , Bluffton, Indiana) and then tempered to 13 ± 0.5% moisture by either adding water or by ambient drying Whole wheat flour was added to each sample of wheat at a rate of one percent by weight to stimulate oviposition by *R. dominica*. Wheat moisture contents were determined using the ASAE standard dry weight method (Society of Agricultural Engineers 1996).

Experiments were conducted with 75.0 ± 1.0 g of wheat in 0 2366 liter round glass canning jars. Forty, 2 − 3 week old unsexed *R dominica* adults, from OSU stock cultures reared on the hard red winter cultivar '2163', were added to each jar for a four-day ovipositional period All jars were kept at 30.0 ± 0.5℃ in an environmental chamber (Percival-Scientific Corp., Model I-35 LVL) during the initial ovipositional period in order to begin with an approximately equal number of eggs in each jar. Following the ovipositional period, grain was gently sieved over a 1.40 mm standard dockage sieve. Beetles were collected below that sieve on a 0.21082 mm sieve while the dust fell through both sieves to be collected in a bottom pan. Wheat and dust were returned to the jars for the duration of the experiment. Ventilation was permitted through filter paper held in place by the canning ring. Samples were maintained at either 27.0 ± 0.5℃ or 34.0 ± 0.5℃ and 70 ± 5% rh. Completely dark conditions were utilized throughout the experiment Hygrothermographs were maintained inside each environmental chamber to monitor any changes in temperature or humidity

Experiments were conducted in a split plot arrangement in which the main plot was temperature, arranged in a completely randomized design, while the subplot treatments were cultivar and presence of the parasitoid, arranged in a randomized complete block. Subplot treatments were blocked across the environmental chambers from left to right because an earlier uniformity trial indicated differences in a chamber Each environmental chamber represented one application of 'temperature' while subplot treatments were each replicated four times within each chamber. The experimental unit at the subplot level in this experiment was a jar of wheat infested with *R dominica*. Within a subplot replication each wheat cultivar was represented two times, once in the absence of the parasitoid and once with the parasitoid present. Each experiment included two replications of temperature (two chambers at each experimental temperature), eight replications of each cultivar with parasitoids per temperature, and eight replications of each cultivar without parasitoids.

Time of beetle and parasitoid introduction and removal times are summarized in Table 1. All adult *T elegans* were less than three days old when released into the grain. Parasitoids were reared in stock colonies on *R dominica* in

'2163' wheat Five male and 10 female parasitoids were released at each release time. Previous research (Toews, unpublished) indicated that generation time for *R dominica* varied among wheat cultivars Due to the possibility of a single parasitoid release being optimally timed for a given cultivar containing the appropriate host stage, two parasitoid releases were utilized. This method insured that live parasitoids were present when their hosts reached the optimum age for parasitism Differences in *R dominica* developmental times at the two different experimental temperatures were estimated with a degree day model developed by Subramanyam et al. (1990). The model used a threshold temperature of 13.2℃ for egg to adult development of *R dominica*. All insect introduction and removal times were calculated to occur at the same degree day intervals in both temperature treatments. Data analysis was performed only on the total number of progeny collected and not broken down by individual collection interval

Table 1. Introduction and removal times for R dominica and T elegans

Temperature	*T. elegans* Released		*T. elegans* Released		*T elegans* Removed		*R. dominica* and *T. elegans* removed		*R dominica* and *T elegans* removed	
	DD[a]	Day[b]	DD	Day	DD	Day	DD	Day	DD	Day
27.0℃	343.2	24	453.6	32	564.0	40	771.0	55	978.0	70
34.0℃	337.6	17	441.6	22	566.4	28	774.4	38	982.4	48

[a] Degree Days based on a model by Subramanyam et al (1990) [b] Actual Calendar day of experiment starting with adult *R dominica* release when all jars were held at 30 0℃ for four days accounting for the first 67 2 degree days in this table; after the four-day ovipositional period the adult *R dominica* were removed and jars moved to their respective temperatures If 24 actual calendar days are shown in this table then the first four days were at 30 0℃ and the other 20 days are at the experimental temperature

Data were analyzed using SAS (SAS Institute Inc 1994). Before statistical analysis progeny counts were transformed using a square root transformation while percentages were transformed using the arcsine transformation (Zar 1984) to correct for heteroscedastic data Treatment means were compared at the $\alpha = 0.05$ level using the LSD method (PROC GLM, MEANS LSD LINES) (Steel and Torrie 1980) Parasitoid counts were analyzed using PROC GLM; however, this method does not account for variability at the first trophic level from a cultivar that harbors only a few beetles and consequently only a few parasitoids. To relate the number of parasitoids emerged to cultivar effects, a 'parasitoid progeny index' was created. This index was determined by dividing the total number of parasitoids emerged from each treatment jar by the total number of beetles emerged from the control jar of the same cultivar in the same replication This index was needed to fully quantify the tritrophic nature of the study When using the parasitoid production index, numbers of *T elegans* are related to the number of emerged beetles in the wheat without parasitoids and therefore the effect of cultivar on beetle numbers is adjusted appropriately Parasitoid progeny index data were analyzed using PROC GLM (SAS Institute Inc. 1994) and were not transformed Values for percent suppression of *R dominica* caused by *T. elegans* were obtained by dividing the number of *R dominica* adults from the jar with the parasitoids present by the number of adults from jars without the parasitoids in the same replication. The resulting number from that calculation was then subtracted from one and multiplied by 100

Results

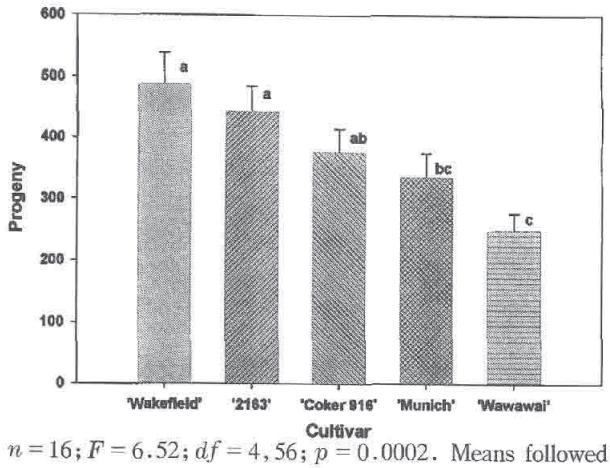
Temperature was not a contributing factor to variability in the number of *R. dominica* adult progeny recovered in grain without *T elegans* ($F = 0.24$; $df = 1,2$; $p = 0.6733$) At 27.0℃, a mean of 358.7 ± 29.6 beetles emerged, while at 34 0℃, 398.1 ± 26.6 beetles emerged per jar of wheat Numbers of *R. dominica* progeny in grain without *T elegans* significantly varied due to wheat cultivars ($F = 6.52$; $df = 4,56$; $p = 0.0002$) (Fig. 1) Total numbers of adult progeny by cultivar ranged from 248.9 ± 27.5 beetles to 487.4 ± 51.4 beetles per jar. The cultivars 'Wawawai' and 'Munich' harbored fewer numbers of *R dominica* than the remaining cultivars

The higher temperature had a significant influence on the suppression of adult *R dominica* by *T elegans* when compared with the lower temperature ($F = 17.78$; $df = 1,2$; $p = 0.0519$) At 27℃ the number of R dominca suppressed were 81.5 ± 3 1% whereas 51.3 ± 5.7% were suppressed at a higher temperature. The were no differences in percent suppression of *R dominica* progeny attributable to the different cultivars ($F = 1.72$; $df = 4, 55$; $p = 0.1580$) (Fig. 2) Suppression of *R dominica* ranged from a minimum of 56.9 ± 8.2% in 'Wawawai' to a maximum of 73.3 ± 8.5% in '2163'

Temperature had an important effect on the recovery of adult parasitoid progeny ($F = 9.94$; $df = 1, 2$; $p = 0.0876$) While a mean of only 24.2 ± 5.3 *T. elegans* emerged from wheat samples held at 34.0℃, a mean of

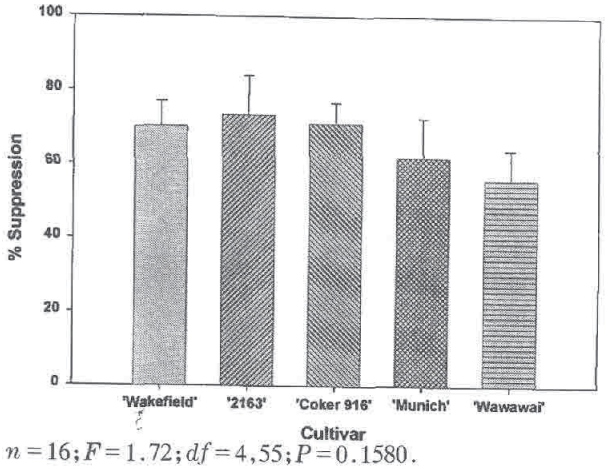
206.5 ± 16.0 parasitoids emerged from grain held at 27.0℃. Fewer *T. elegans* progeny emerged from 'Wawawai' than from the remaining cultivars (Fig. 3). This was expected since this cultivar also harbored the fewest number of *R. dominica*.

The parasitoid progeny index was significantly higher at 27.0℃ than at 34.0℃ ($F = 19.93$; $df = 1, 2$; $p = 0.0467$). At the higher temperature the mean parasitoid progeny index was only 0.11 ± 0.03 while at 27.0℃ it was 0.69 ± 0.09. This index was not influenced significantly by wheat cultivar ($F = 1.80$; $df = 4, 56$; $p = 0.1416$) (Fig. 4). Values for the index averaged over both temperatures ranged from a minimum of 0.27 ± 0.07 parasitoids per available host in 'Wawawai' to a maximum of 0.58 ± 0.20 parasitoids per available host in 'Munich'.



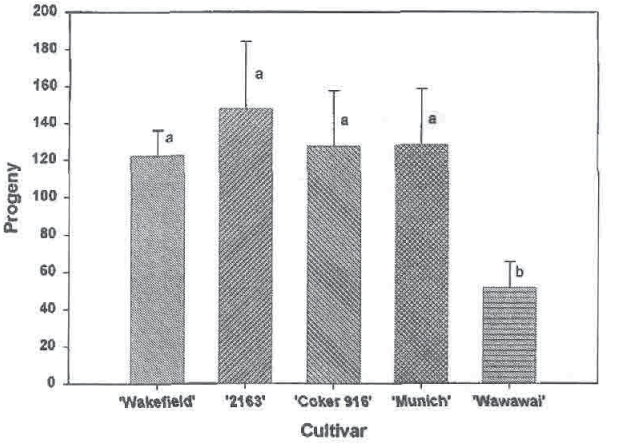
$n = 16$; $F = 6.52$; $df = 4, 56$; $p = 0.0002$. Means followed by the same letter are not significantly different ($p > 0.05$; Fishers LSD Test).

Fig. 1. Mean ± SE *R. dominica* adult progeny from wheat without *T. elegans* present.



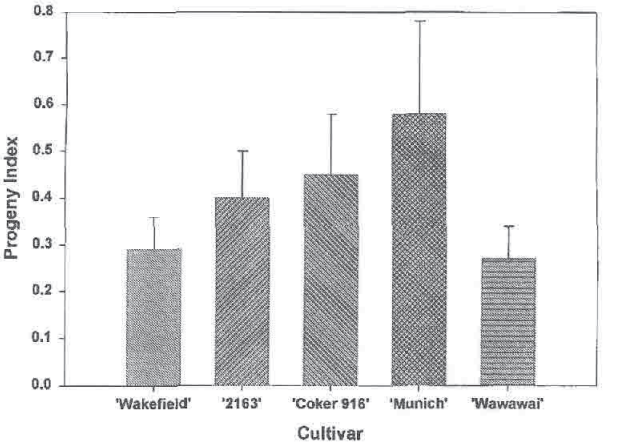
$n = 16$; $F = 1.72$; $df = 4, 55$; $P = 0.1580$.

Fig. 2. Mean percent ± SE suppression of recovered *R. dominica* adult progeny when parasitized by *T. elegans*.



$n = 16$; $F = 10.52$; $df = 4, 56$; $p = 0.0001$. Means followed by the same letter are not significantly different ($p > 0.05$; Fishers LSD Test).

Fig. 3. Mean ± SE *T. elegans* adult progeny.



$n = 16$; $F = 1.80$; $df = 4, 56$; $p = 0.1416$.

Fig. 4. Mean ± SE *T. elegans* progeny production index.

Discussion

The functional response concept is a useful tool to help explain differences in beetle suppression between these temperatures. The functional response is defined as the change in the number of hosts consumed or parasitized (stung) by individual predators or parasitoids as the density of the prey/host changes (Solomon 1949, Holling 1959). Later, Mack and Smilowitz (1982) suggested that search rate, a component of the functional response, decreased in relation to increasing temperatures above 30.0℃ for the predator *Coleomegilla maculata* (DeGeer). (Coleoptera: Coccinellidae). In this experiment, the functional response describes the number of *R. dominica* stung by *T. elegans*. An increase in the search and handling time due to the higher temperature, could have caused the lower rate of

suppression of the host, since there were not enough parasitoids to sting the number of available hosts. At the lower temperature, the parasitoids were able to attack their hosts more quickly and likely parasitized a higher proportion of the pest population These data imply that temperature is an important component of the functional response for *T. elegans*.

In this experiment, the 34.0℃ temperature significantly decreased the recovery of adult parasitoid progeny. Possibly, the high temperature prohibited a certain physiological event from occurring in the life cycle of the parasitoid. Another hypothesis is that hosts at the high temperature are being stung, thus paralyzing the beetle larvae, but adult female parasitoids are not depositing eggs; this behavior has been shown with another species of stored grain parasitoid (Flinn 1991)

Solomon (1949) described the numerical response, defined as a change in the number of predators, as a function of prey density from generation to generation Entomophagous insects in biological control studies usually control pest populations through changes in the numerical response (Huffaker et al. 1968). The current experiment does not include conclusions on the numerical response of the parasitoid because these conclusions would require data from several generations. However, in both of these experiments, significantly more *T. elegans* emerged per available host at the lower temperature than at the higher temperature Assuming that host density is not a limiting factor and that more parasitoids are able to parasitize an increased number of hosts, the potential for a numerical response should increase at 27.0℃, compared to 34.0℃. Additional research targeting the numerical response for this parasitoid is needed to address these possibilities.

Cultivar played a limited role in the association among trophic levels. Differences in *T. elegans* progeny recovered among cultivars were linked to the increased number of *R dominica* hosts in those same cultivars. The parasitoid progeny production index provides more information on the influence of the wheat cultivar on the parasitoid A higher parasitoid production index value, such as that calculated for 'Munich' suggested that the first trophic level is positively influencing the third trophic level. More parasitoids emerged per potential host on the cultivar 'Munich' than in 'Wawawai', however cultivar was not a statistically significant factor contributing to differences in the index value in either experiment.

Conclusions

T. elegans played an important role in suppressing progeny of *R. dominica*. Suppression of the second trophic level (*R. dominica*) by the third trophic level (*T. elegans*) will prevent excessive losses at the first trophic level (grain). High temperature significantly decreased *T. elegans* progeny recovery. Efficacy of the parasitoid and the number of parasitoid progeny were both increased at the lower temperature, potentially increasing the opportunity for successful control. The first trophic level, wheat cultivar, did not significantly affect the parasitoid production index but did affect the recovery of *R. dominica* progeny in wheat without *T. elegans*. There were no differences attributable to temperature in the number of *R. dominica* progeny recovered in grain without *T. elegans*.

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