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(Received 2 February 2013, revised 6 March 2013)

Pakistan J. Zool., vol. 45(3), pp. 874-878, 2013.

Costs of Resistance to Insecticides in the Maize Weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae)

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Abstract. *Sitophilous zeamais,* the maize weevil is a main cause of infestation in stored maize throughout the tropical regions of the world. The attacked seeds are damaged badly, reduced their weight, nutritional values and market value. For the protection of maize crop, farmers usually use pyrethroid and organophosphate insecticides. The indiscriminate use of these insecticides resulted in the development of resistance in Maize Weevil and affected their growth rate. Therefore for the determination of effect of insecticides on life history of *Sitophilous zeamais,* two pyrethroid-resistant strains from fields of the counties Juiz de Fora (JF) and Jacarezinho (JA) from State of Parana were compared with one strain susceptible to both insecticides from the county of Sete Lagoas (SL), which were reared in an insectary, under the controlled condition. We tested whether individual-based life histories of two strains of the maize weevil *S. zeamais* resistant to pyrethroid insecticides showed fitness costs relative to a susceptible strain. None were detected, despite such costs having been shown in a mass-rearing experiment using the same strains.

Key words: Insecticide resistance, maize weevils.

A major cause of losses to stored maize throughout the tropical and temperate regions around the world is the maize weevil *Sitophilous zeamais* Motschulsky 1855 (Coleoptera: Curculionidae). Damaged grains have reduced nutritional values, low percent germination and reduced weight and market value. Worldwide seed losses ranging from 20-90 % have been reported for untreated maize due (Giga and Mazarura, 1991). Pyrethroid and organophosphate insecticides are heavily used for maize weevil control (Fragoso *et al.,* 2003; Guedes *et al.,* 1995), and development of resistance is a serious worldwide challenge for pest management of such stored–product pests. Over-reliance on insecticides for controlling the maize weevil and other stored grain pests in tropical areas has made insecticide resistance a frequent problem (Champ and Dyte, 1976)

Insecticide resistance affects weevil physiology (Fragoso *et al.,* 2007) and components of the life history that map into population growth rate (Fragoso *et al*., 2005). The paradigm of such effects involves the idea that there is a cost to resistance, but in environments with insecticides the benefits outweigh the costs and resistance evolves (Baucom and Maurizio, 2004). The same paradigm is used in

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interpreting insect resistance to plant secondary defensive chemicals (Zavala *et al.,* 2004), such as the furanocoumarins of umbellifers (Strauss and Zangerl, 2002). In the absence of herbivores, plants producing such chemicals are at a selective disadvantage. Thus insecticide resistance should disappear when insecticides are withdrawn, since then the costs outweigh the benefits, which are not expressed in an insecticide-free environment (Baucom and Maurizio, 2004). This is exactly what happens with the resistant weevil strains used here (Guedes *et al*., 1995; Ribeiro *et al*., 2003; Fragoso *et al*., 2003, 2007).

Fragoso *et al.* (2005) studied the population growth of *S. zeamais,* assessing two resistant and two strains susceptible to pyrethroid pesticides. Using mass culturing methods, they found that both susceptible (from Bragança Paulista and Sete Lagoas) and one resistant strain (from Jacarezinho) were very similar to one another, while a further resistant strain (from Juiz de Fora) showed reduced developmental rate, delayed emergence, higher mortality and reduced growth rate. The main object of this study was to evaluate differences among three of these strains using individual-based data, to dissect further the relevant life-history differences.

Materials and methods

Three strains of *S. zeamais* were used in this study, originally collected from Brazil: two pyrethroid-resistant (>100-fold in bioassays) strains from fields of the counties Juiz de Fora (JF, from the State of Minais Gerais in 1999: Guedes *et al.,* 1995; Fragoso *et al.,* 2003) and Jacarezinho (JA, State of Parana in the late 1980s), and one strain susceptible to pyrethroids and organophosphates (Guedes *et al.,* 1994, 1995; Fragoso *et al.,* 2003; Ribeiro *et al.,* 2003; Araujo *et al.,* 2008) from the county of Sete Lagoas (SL, State of Minais Gerais, maintained for 20 years without exposure to insecticides in the National Research Centre of Maize and Sorghum of the Brazilian Agricultural Research Corporation, Embrapa Milho e Sorgho). All strains were kindly provided by Dr. Guedes and Rubia Araujo from the Ecotoxicology Laboratory of the Federal University of Viçosa, Minais Gerais, Brazil.

The deltamethrin-resistance of the JA strain is sex-linked (Guedes *et al.,* 1994, 1995; Ribeiro *et al.,* 2003). The JF population shows an overall fitness disadvantage in the absence of pyrethroids, unlike the JA population (Fragoso *et al.,* 2005; Oliveira *et al.,* 2007). All populations were maintained in mass-culture jars on whole maize grains (13% moisture content) at a temperature 25±2°C.

Weevils were reared at 27±2°C (in an insectary of School of Biology, University of Nottingham, Nottingham, UK) in male-female pairs in Petri dishes of 90 mm diameter with filter paper on the bottom, and provided with ten insecticide–free whole maize grains as feeding and oviposition substrates. The sexes were identified by the shape of their snout. Petri dishes were kept in boxes over salted water for maintaining humidity, in an insectary at 25-27°C.

Ten newly emerged male-female pairs were selected at random from stock cultures of each strain. Every day for three months from 6th January 2010 to 7th April 2010, each pair was transferred to a new dish: the number of eggs laid in the maize grains of the old dish were counted, and the dish placed in a rearing box to await the emergence of the adults. Emergence was assessed every day. After the 7th April, pairs were simply maintained until death, without measuring egg production any more, or until the end of June 2010 when the experiment stopped.

Fewer eggs were counted than were laid because ovipositions were difficult to see. Females bite a small hole, lay an egg and coat it with a glycosidic substance that hardens rapidly into a solid egg plug that seals the hole (Woodbury, 2008). Often this is hardly any different from the maize surface, and in addition the hole is often drilled around or under imperfections in the surface, making some all but impossible to see. Thus the number of eggs counted does not match the number of emergences recorded, and very often the latter is greater than the former.

Statistical analysis was carried out using R (Zuur *et al*., 2009). All variable residuals were checked for normality, and non-parametric tests used where transformations (log or square-root) were unsuccessful in resolving the non-normality. KW indicates a Kruskal Wallis non-parametric test for one-way group differences, distributed as a χ^2.

Results

About 50% more eggs were counted from females of the JA strain (1408) than either JF (961) or SL (936) females, mostly because fewer females failed to produce eggs rather than higher production per female (Fig. 1): the differences were not significant (KW=2.8, df=2, ns). The JA strain also produced more emerging adults (1690) than either JF (930) or SL (956), but again the values for each pair were very variable, and the means for the strains were not significantly different (KW = 4.2, df=2, ns). There were no differences in longevity among strains for males (KW = 2.0, df=2, ns) or females (KW = 2.5, df=2, ns).

To demonstrate the pattern of egg laying, we plotted the mean number laid per week for each strain (Fig. 2). Notice how the number rises rapidly to a peak, and then declines more or less linearly to reach zero or near zero by week 14. We therefore removed data from before day 10 and after day 90 to restrict them to the period within which the relationship between eggs laid and laying day is approximately linear. The number of eggs counted per day was then analysed by a generalised linear model with quasi-Poisson errors in a model consisting of pair-within-strain, strain, laying day (covariate) and an interaction between laying day and strain (*i.e.* a test for different slopes for the strains). Much of the deviance was accounted for by pairs-within-strains (Wald χ^2_{25} = 329.3, p<<0.001) and the overall regression with laying day (Wald χ^2_1 = 263.8, p<<0.001) with its negative slope. While there were no differences overall among strains (Wald χ^2_2 = 3.0, ns), there were significant differences among the strains in the slope of the relationship with laying day (Wald χ^2_2 = 14.5, p=0.001), all accounted for by the slope of strain JA (m = -0.015±0.0056) which was shallower than those of SL (-0.025 ± 0.0025) and JF (-0.023 ± 0.0058).

Egg-to-adult development times varied from 28 to 50 (Fig. 3), with a mean of 36.3 ± 1.3 days. In a mixed model of the effects of pair-within-strain, strain and laying day on development times for individual weevils, there was no evidence of any differences among strains ($F_{2,19.2}$ = 0.22, ns), but a strong negative effect of laying day ($F_{1,3533}$ = 63.5, p<0.001; slope = -0.0246±0.0031) with no evidence for strain differences in slopes ($F_{2,3410}$ = 2.07, n.s.). The slope indicates that over the laying period of approximately 100 days, the egg-to-adult developmental period declined by about 2.5 days (Fig. 4).

The pattern of number of emergences from eggs laid on any one day was similar to that of the number of eggs. We similarly restricted the analysis to laying days after 10 and before 90. There were no strain differences overall (Wald χ^2=2.9, df=2, ns), but there was a strong negative effect of laying day (Wald χ^2=207.3, df=1, p<<0.001) with significant differences among strains in the slopes (Wald χ^2=22.5, df=2, p<0.001). Once again the JA strain had a shallower slope (-0.010) than the other two strains (SL, -0.021; JF -0.019).

Discussion

Maize weevil resistance to pyrethroid insecticide has been linked with reduced target-site sensitivity as the major mechanism, and increased detoxification by glutathione-S-transferases as a secondary mechanism (Fragoso *et al.*, 2003, 2007; Guedes *et al.*, 1995, 2006; Ribeiro *et al.*, 2003). The target site resistance was sex-linked (Guedes *et al.*, 1994). Both resistant strains of the weevil were heavily subjected to pyretheroid insecticide application.

Fragoso *et al.* (2005) found that the Juiz de Fora (JF) population showed a fitness disadvantage in the absence of pyretheroids unlike that of Jacarezinho (JA). Based on mass data, they found that the JF population had reduced and delayed emergence relative to susceptible strains (Fragoso *et al.*, 2005; Oliveira *et al.*, 2007). Looking at individual-based data, we failed to detect any such reduced or delayed emergence in the JF strain. There were no differences of any kind between the resistant JF strain and the susceptible SL strain. The only possible differences among strains lay in the slightly more gently decline with female age in the number of eggs laid and the egg-to-adult development time of her offspring in the resistant JA strain relative to the other two. Although logistic reasons prevented us from using large numbers of pairs, and so statistically we are not sure, it is certainly possible that JA females are less likely to fail to lay.

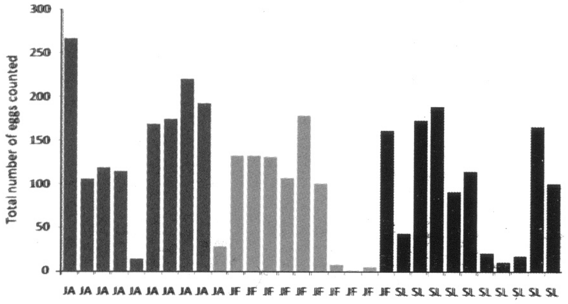

Fig. 1. Total number of eggs counted for each pair of the three different strains. (JF, Juiz de For a (resistant); JA, Jacarezinho (resistant); SL, Sete Lagoas (susceptible).

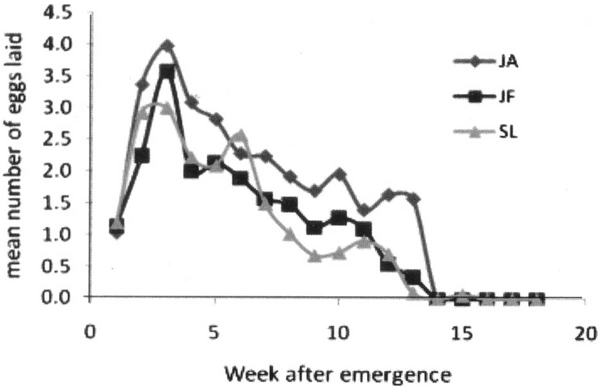

Fig. 2. The number of eggs laid per week by pairs of *Sitophilus zeamais* weevils of three different strains. For abbreviations see Figure 1.

Fragoso *et al.* (2005) did not find differences between the resistant JA strain and susceptible strains, suggesting that resistance might be fixed in this laboratory population, accounting for its relatively good demographic performance. They also pointed to the good conditions of laboratory culturing. It is certainly true that demographic trade-offs might only be evident under stressful conditions (Zera and Harshman, 2001), unlike those of the laboratory environment. Thus if we were to isolate weevils without food for periods of time, then such costs might be measurable.

Acknowledgements

We thank Dr RNC Guedes and Rubia Araujo from the Ecotoxicology Laboratory of the Federal University of Viçosa, Minais Gerais, Brazil for the strains of *Sitophilus zeamais* used in these experiments. We thank the Higher Education Commission of Pakistan for funding to Professor Memon.

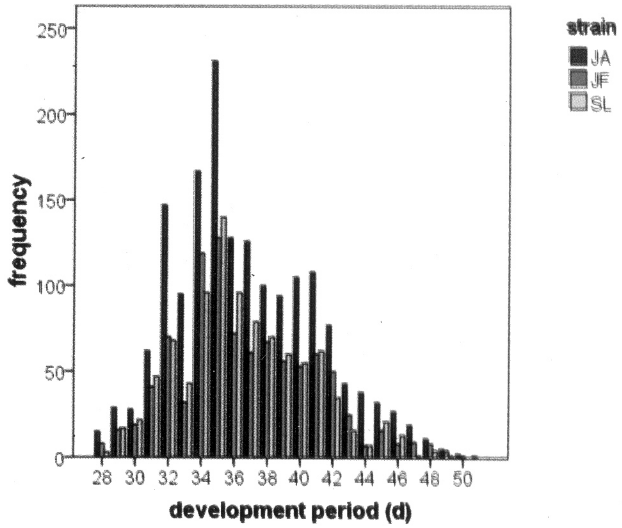

Fig. 3. Frequency distribution of the egg-to-adult developmental period for the three strains of *Sitophilus zeamais*. For abbreviations, see Figure. 1.

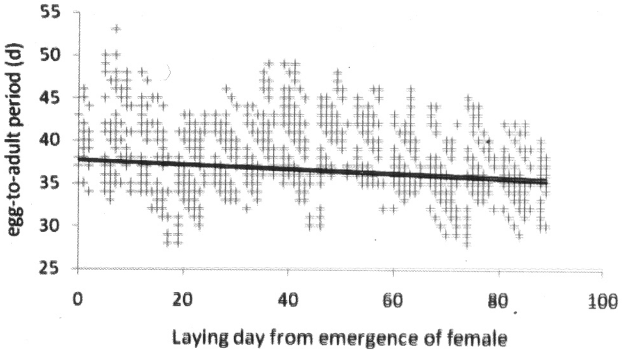

Fig. 4. Relationship between egg-to-adult developmental period (in days) and laying day (from emergence of female).

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(Received 6 February 2013, revised 25 March 2013)

Pakistan J. Zool., vol. 45(3), pp. 878-881, 2013.

Consequences of Spring Warming for the Black Redstart (*Phoenicurus ochruros*) in N.W. Croatia

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Abstract.- Average global temperatures in Earth have increased over the past 100 years and climate change impacts wildlife in countless ways. Numerous of the works demonstrated climatic impacts on bird phenology. We used 26 years of data from black redstart (*Phoenicurus ochruros*) in population from Mokrice village (northwestern Croatia), 1987-2012. Correlation between timing of arrivals and year was significant. Black redstart arrival dates have become 8.84 days earlier during the research period. The relationship between local mean spring temperatures and year was significant. The date trends in spring migration correspond with increasing mean spring air temperatures (March-April). This result suggests that black redstart respond to air spring temperatures by earlier arrival at breeding grounds. Our results are consistent with many other long-term studies of the timing of birds migration and suggest that air spring temperature change may be responsible for shifts in arrival dates.

Key words: Spring temperatures, black redstart, Croatia

Average global temperatures in Earth have increased over the past 100 years (IPCC, 2007), and climate change impacts wildlife in countless ways (Parmesan, 2006). For example, studies from throughout the world have demonstrated that tree

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