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Preliminary results on impact of nitrogen fertilisation on *Sitophilus zeamais* wheat-food preferences and progeny production

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Abstract

Preliminary results on impact of nitrogen fertilisation on the maize weevil (*Sitophilus zeamais* Motschulsky) wheat-food preferences and progeny production are reported. The Italian durum wheat (*Triticum turgidum* L.) variety "Svevo" was cultivated using three different fertiliser regimes, 0, 60, and 160 kg/ha of nitrogen. The kernel samples obtained by the three fertilisation regime-plots were compared. After bioassays were performed in cylindrical arenas, different levels of kernel susceptibility to *S. zeamais* infestation were observed. The investigations indicated that the susceptibility of grains is not closely related to higher protein presence in kernels from field plots fertilised with 60 and 160 kg/ha of nitrogen compared to the kernels of unfertilised plots. These observations were consistent with the results observed in F1 and F2 progeny. The overall duration of developmental time of *S. zeamais*, from egg to adult, was also not influenced by the different nitrogen fertiliser regimes.

Key words: maize weevil, nitrogen fertilisation, progeny production, wheat-food preferences.

Introduction

Potential crop yield is determined by environmental aspects, such as the length of growing season, rainfall, temperature regime, and soil characteristics. The producer must apply agronomic practises that affect cultural selection, seeding timing, depth and density of planting, tillage practises, crop rotation, irrigation, control of weeds, diseases, and pests, and nutrient management to capture as much yield potential as possible while supporting long-term environmental sustainability.

In the majority of cases, the nutrient nitrogen limits durum wheat production and it is applied in the greatest amount. It is a component of plant proteins, amino acids, nucleotides, nucleic acids, and chlorophyll. Therefore, nitrogen is essential for both optimum crop yield and optimum quality, as protein production is directly related to nitrogen supply (Grant and Flaten, 1998).

Grain protein recently has received attention in several countries due to a premium associated with increased protein concentration in grains. Nitrogen fertiliser management, specifically timing of application and rates, plays a crucial role in increasing protein concentration. However, the economically optimum nitrogen fertilisation amount will vary depending on grain prices, fertiliser costs, and yield potential. Therefore, there is a need to adopt agronomic and economic models that determine the optimum nitrogen rate and timing and are useable by growers and their advisers to achieve higher yield and grain-protein concentrations (Colecchia *et al.*, 2013).

However, due to economical and ecological reasons, fertilisation should be conducted carefully in order to apply only the amount necessary for optimal pant growth (EC Nitrate Directive 91/676/EEC). Several studies have demonstrated that excessive nitrogen application, especially in cereal cropping systems, often leads to nitrogen leaching, which causes soil and water pollution (EC, 1991). Although several research groups have focused on finding optimal nitrogen doses, in order to improve yields and seed quality, we still have little information on the impact of fertilisation on wheat insect pests, particularly on stored product pests, wheat diseases, or other organisms associated with these cropping systems.

According to Mattson (1980) and Ritchie (2000), the application of nitrogen fertiliser to plants normally can increase herbivore feeding preference, food consumption, survival, growth, reproduction, and population density, with the exception of a few examples. High nitrogen levels in plant tissue can decrease resistance and increase susceptibility to pest attacks (Mebarkia *et al.*, 2010; Veromann, 2013). Crops can be expected, therefore, to be less prone to insect pests and diseases where organic soil amendments are used, since these amendments usually result in lower concentrations of soluble nitrogen in plant tissue (Lampkim, 1990; Altieri *et al.*, 1998).

Only a limited number of studies have focused on the effects of nitrogen fertilisation on parasitism level of stored insect pests (Veromann *et al.*, 2013; Demissie *et al.*, 2015)

In this paper, the impact of nitrogen fertiliser on maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera Dryophthoridae) wheat-food preferences and progeny production in F1 and F2 generations is reported. Kernels of the Italian durum wheat (*Triticum turgidum* L.) variety "Svevo" from fertiliser regimes with 0, 60, and 160 kg/ha of nitrogen were compared.

S. zeamais is universally considered one of the most important pests of stored cereals and is present in the warmest and tropical parts of the world. Voracious feeding on whole grains by these insects causes weight loss, fungal growth, and loss of quality through an increase of free fatty acids. The consequences can even result in complete destruction of the stored grain in all types of storage. Invasion by this primary pest may facilitate the establishment of secondary insects, mite pests, and plant pathogens. Where grain is stored on small farms, *S. zeamais* is able to fly to the ripening crop in the field and establish an infestation in the grain before harvest (Giles, 1969; Longstaff, 1981; Trematerra and Throne, 2012).

Materials and methods

Experimental field plots

The field studies were conducted in 2012 at a traditional farm of about 10 hectares located 700 m above sea level in a hilly area of the Molise region (Central Italy). Three different nitrogen fertiliser levels per hectare were considered on three randomised replicate plots of 10 m^2 (1.5 × 6.67 m). Treatments applied included unfertilised plots and those fertilised with nitrogen applications, either 60 or 160 kg/ha (hereafter indicated as N0, N60 and N160, respectively), equally divided among sowing [as diammonium phosphate 18-46, $(NH_4)_2HPO_4$], tillering (as ammonium nitrate, NH_4NO_3), and stem elongation [as urea, $CO(NH_2)_2$].

The experiment was conducted using Italian durum wheat (*T. turgidum*) of the variety "Svevo"; during the tests, no insecticides, fungicides, or herbicides were applied.

The protein contents in the kernel samples from plants in the experimental plots were estimated using the total nitrogen content (%) according to the Kjeldahl method [total Kjeldahl nitrogen (TKN)], described in AOAC (1995).

Insects

Newly emerged, younger than 12-h-old adults of *S. zeamais* were collected with rearing glass jars containing maize; weevils were a laboratory strain originating from Central Italy. These *S. zeamais* individuals were reared for four generations on maize. The beetles were sexed individually according to their pronotum characteristics (Nardon and Nardon, 2002) and shape of their rostrum, which is distinctly longer, narrower, and smoother in females compared to males (Halstead, 1963). Afterward, males and females were kept separately in 30-mL plastic cups until testing started. The weevils had no access to food or water and were kept under the same odour conditions.

Bioassays

The bioassays were performed in cylindrical arenas made of Plexiglas, each with a diameter of 45 cm and height of 30 cm. Teflon paint was used at the upper internal parts of the arenas to prevent beetles from escaping (Trematerra *et al.*, 2000). Three Petri dishes, each with a diameter of 9 cm and height of 12 mm, were placed in each test arena (three-choice tests). Each dish contained 136 g (about 3150 grains) of whole kernels (13% moisture content) of the durum wheat variety "Svevo" cultivated under the three different fertiliser regimes (N0, N60 and N160).

The positioning of the three dishes (choices) was randomised prior to each bioassay. In every trial, 10 adults of *S. zeamais*, five virgin males and five virgin females, were released carefully in the middle of the arena. Five replications were performed for each test, using a total of 50 specimens (25 males and 25 females). After 18 d, considered as oviposition period, the adults were removed from the arenas.

The grains of each Petri dish were separated into 15 different glass jars (five jars each of N0, N60 and N160); in each jar were added 200 g of the same uninfested wheat. The 15 jars were incubated at 28 ± 1 °C, 70 ± 5% RH. After 35 days, the newly emerged adults were sieved off every 3-4 d (Danho *et al.*, 2002). The weevils emerging from each jar were counted to provide a measure of F1 productivity. All progeny production was determined and compared with the three nitrogen fertilisation levels, as was the average time to adult emergence. Data also were collected on F2-weevil progeny (as function of F1), which involved different jars.

All rearing and experiments were conducted at 28 ± 1 °C, 70 ± 5% RH in continuous darkness.

Data analysis

The statistical analysis was performed using a one-way analysis of variance (ANOVA), where the independent variable was the nitrogen fertilisation regime. To normalise the distribution, data were natural-log (x + 1) transformed. The homogeneity of variances was assessed by the Levene statistic. In addition, the Spearman's rank correlation test was also assessed on row data. All analyses were done using SPSS software version 13.

Results

According to the Kjeldahl method, the protein contents in the kernel samples of N0, N60 and N160 were different. The variation in protein contents included the following: protein from kernel samples of N0 = 8.9 ± 0.5% of the dry mass; protein from kernel samples of N60 = 10.5 ± 0.9% of the dry mass; protein from kernel samples of N160 = 15.4 ± 1.0% of the dry mass.

S. zeamais adults emerged after incubation from samples of the three different grain-production field plots (N0, N60 and N160) and are reported in figures 1 and 2.

Results obtained in our experiments showed that maize weevil adults have differing degrees of preference for attacking samples of the three grain-production field plots (figure 1).

Overall, from the three wheat samples, 804 adults emerged during F1 progeny, and 1470 adults emerged during F2 progeny (figure 1). During F1 progeny, 20.15% of total adults were obtained from samples of N0 plots, 33.58% of total adults were obtained from samples of N60 plots, and 46.27% of total adults were obtained from samples of 160N plots (figure 1). In different jars, F2-progeny-production generation produced 18.95% from N0, 31.08% from N60, and 49.47% from N160 treatments (figure 1).

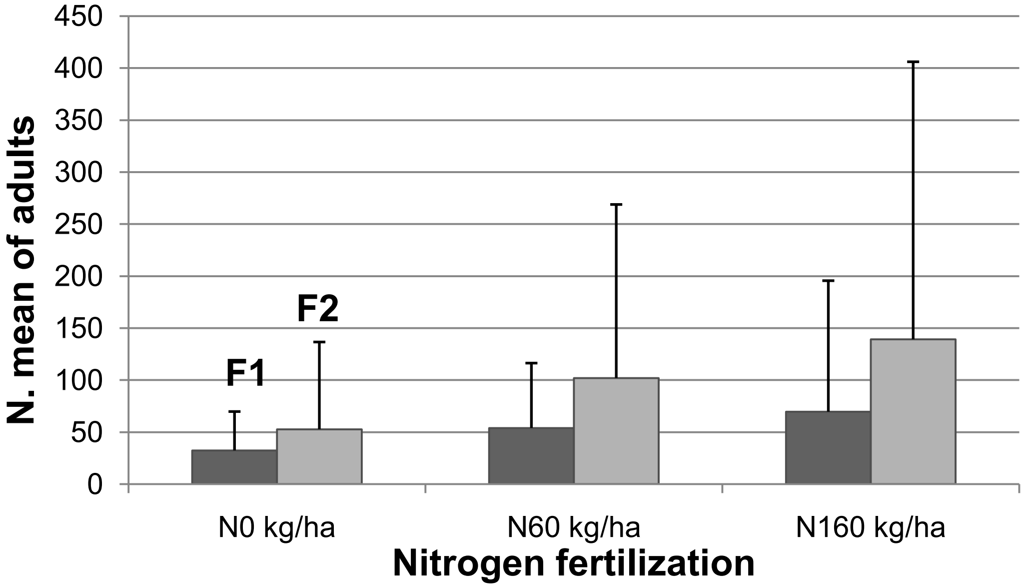

Figure 1. Means of *S. zeamais* adults that emerged (± SD) from durum wheat kernels fertilised with different quantities [0 kg/ha (N0); 60 kg/ha (N60); and 160 kg/ha (N160)] of nitrogen (F1 and F2 progeny).

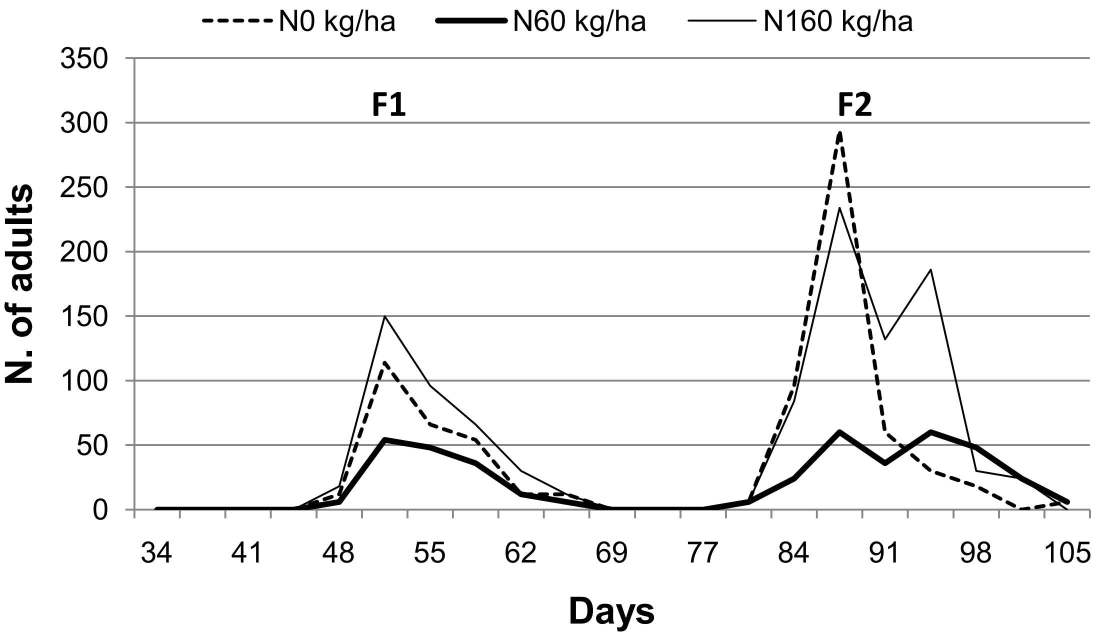

Figure 2. *S. zeamais* adults that emerged (in F1 and F2 progeny) from durum wheat kernels fertilised with three different quantities of nitrogen [0 kg/ha (N0); 60 kg/ha (N60); and 160 kg/ha (N160)].

Both ANOVA results ($P < 0.05$) and Spearman's rank correlation test, during the entire developmental period, from egg to adult, F1 and F2 progeny production was not significantly influenced by the three different nitrogen fertiliser levels (figure 1).

From the three different sample types, adults of the F1-progeny-production generation first emerged after 48 d; then, during the next 18 d and up to 66 d after the beginning of the experiment, more adults were observed. In the case of F2, *S. zeamais* adults emerged consecutively for 25 d, starting after 32 d (80 d after the start of the experiment) and up to 24 d (105 d after the experiment began) (figure 2).

Discussion

As reported, in the open field, the application of nitrogen fertiliser to plants can increase insect feeding preference, food consumption, survival, growth, reproduction, and population density. High nitrogen levels in plant tissue can decrease resistance and increase susceptibility to pests (Mebarkia *et al.*, 2010; Veromann, 2013). According to the results of our study and referring to the activity of *S. zeamais*, these phenomena do not occur during post-harvest stages.

The results of our study show that nitrogen fertilisation can affect adult *S. zeamais* host selection, wheat-food preferences, and progeny production, albeit not in a simple, linear manner. The numbers of F1 and F2 adult progeny increased in conjunction with the quantity of nitrogen provided, but the differences were not significant. Results of our study show that the attractiveness of wheat kernels is not strictly linked to the nitrogen fertilisation effects on *S. zeamais* damage.

Laboratory tests on the three different kernel samples, compared to the progeny results, suggest that the susceptibility of these grains to *S. zeamais* infestation can be attributed only in part to the increase of protein contents among the N0, N60 and N160 treatments.

Susceptibility to *S. zeamais* of a grain variety is polygenic, indicating that multiple factors are responsible (Khan *et al*., 2014). It is well-known that many insect pests select their hosts based on visual and semiochemical stimuli (Cook *et al*., 2007). In *Sitophilus*, both physical and chemical properties of kernels have been shown to influence the host preference and acceptance of egg-laying females (Trematerra *et al*., 2007; 2013).

Physical features of the pericarp and seed, along with biochemical components of these structures that elicit behavioural responses of stored-product insects, affect the extent to which insects can use this cereal for growth and development (Seitz, 1995; Throne *et al*., 2000; Fouad *et al*., 2013; Demissie *et al*., 2015). Grain size, hardness, protein content, and oil content have been most commonly analysed when evaluating resistance mechanisms in wheat (Chunni Ram, 1996; Stejskal and Kucerova, 1996).

Sitophilus oviposition behaviour is an important contributor to the fitness of insects due to the resultant effects on the number and quality of offspring (Stejskal and Kucerova, 1996). Olfaction is the means by which weevil adults identify grain as a location at which to carry out important life functions, such as finding food or a mate, and oviposition (Trematerra *et al*., 2007; Trematerra, 2013).

Chemical communication between herbivores and their host plants depends on the plant and herbivores species and is generally based on multiple compounds (Blight *et al*., 1997). Germinara *et al*. (2008) showed that the antennae of adults of the granary weevil (*Sitophilus granarius* L.) detected a wide variety of compounds, such as aliphatic alcohols, aldehydes, ketones and aromatics.

Kernels of cereal plants, such as wheat, emit a complex mixture of volatile organic compounds. The attractiveness of wheat to *S. zeamais* can be attributed to the active volatile components released by the grain (Maga, 1978; Trematerra *et al*., 2000). As an example of the difficulties involved in the process, more than 100 volatile compounds have been analysed in wheat samples from Kansas. However, no differences in composition of these volatiles were found among the five cultivars tested (Seitz, 1995).

The number of compounds present in the volatiles emitted from stored grains, as well as their concentrations, change over time (Zhou *et al*., 1999; Sides *et al*., 2001). In this regard, Germinara *et al*. (2008) suggested that host finding by the granary weevil is a complex process that depends on the balance of positive and negative volatile stimuli from the grain, as the relative concentrations of volatiles may change during storage.

Nitrogen fertilisation may affect the composition and levels of these volatiles and, therefore, also affect their attractiveness to pests (Chen *et al*., 2010). Results of our preliminary trials showed a probable difference in volatile compounds and/or their concentrations in wheat obtained by different fertiliser regimes, which confirms several previous observations (Cook *et al*., 2007), but this must be assessed in more specific future chemical studies.

Our bioassay tests, using wheat of the three different fertiliser regimes, suggest that *S. zeamais* adults are able to respond to odours coming from different kernel samples; kernels with no nitrogen treatment (N0) resulted in being less interesting to weevils than kernels treated with low (N60) or high (N160) nitrogen levels. These observations are consistent with the results observed in the progeny average of different wheat plots, and they confirm the correlations between multiplication rate and protein contents of the samples from the three plots. This shows that nitrogen fertilisation can modify the susceptibility of the wheat to *S. zeamais* attack during storage. Additional studies could provide more information about this relation. In this regard it is useful to remember that the number of adults emerging from any given host can be influenced by the nutrient quality of the host as well as host defences which may play a role in mortality of eggs or newly emerged larvae (Dicke, 1999; Trematerra *et al*., 2007; Chen *et al*., 2010; Dicke and Baldwin, 2010; Demissie *et al*., 2015). As reported by Dinuta *et al*. (2010) in regard to the congeneric *S. granarius*, another reason for an only moderate influence of nitrogen fertilisation on the numbers of *S. zeamais* offspring could be that the minimum larval requirement in protein is fulfilled even in the durum wheat grown without fertilisation.

Following Longstaff (1981), in *Sitophilus*, the length of the life cycle also depends on the type and quality of the grain infested. In our case, in the F1 and F2 generations the developmental time of *S. zeamais* from egg to adult was not influenced by the three different nitrogen fertiliser levels.

Less-susceptible or resistant varieties are of particular interest for the reduction of grain loss in storage (Horbes, 1983; Chunni Ram and Singh, 1996; Suleman *et al*., 2000; Throne *et al*., 2000; Gharib, 2004; Babarinde *et al*., 2008). Currently, the susceptibility or resistance to insect attack during storage is not generally considered during breeding programmes, nor are they evaluated before the release of commercial varieties or during fertiliser programmes. The resistance mechanisms of wheat varieties have not been clarified, and additional studies on plant biogenic volatile involvement remain necessary. Perhaps one of the best ways to promote the development of these aspects in stored products is to link economic losses to the variety used. Varietal susceptibility or resistance to stored grain insects provide the basis on which to build an integrated pest management programme.

Additional studies to be developed, with more pests and possibly also more plant biogenic volatile collections, could provide more information about this insect-host kernel communication system. Consequently, a clear understanding of the biological activity of different cereal volatiles, individually and in combination, will be essential for developing semiochemical-based weevil management strategies (Piesik and Wenda-Piesik, 2015).

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