Bulletin of Insectology **63** (2): 251-258, 2010
ISSN 1721-8861

Insecticide resistance in Italian populations of *Tribolium* flour beetles

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Abstract

As a consequence of the widespread use of chemical insecticides in the control of insect pests in grain warehouses and in the food industry, insecticide resistance in grain insect pests has greatly increased all over the world. The goal of this work was to investigate insecticide resistance levels in Italian populations of *Tribolium castaneum* (Herbst) and *Tribolium confusum* du Val (Coleoptera Tenebrionidae) collected in grain and food storage facilities of 18 different localities. Six contact insecticides were tested on seven populations of the red flour beetle and on eleven populations of the confused flour beetle. Topical application bioassays were carried out on adults. Dose-mortality lines were estimated to determine the resistance ratios for each insecticide and population. A Principal Components Analysis (PCA) was performed on a data matrix describing the pattern of occurrence of the RR values of each insecticide across the set of data provided by 18 different sites. In both species, the slopes of LD-lines range within a rather narrow interval with respect to susceptible strains, while the highest RR value can be observed in a population of the confused flour beetle from Molise assayed with deltamethrin. The distribution of the experimental points in the PCA graph suggests that *T. castaneum* populations were generally more susceptible to malathion, diazinon and pirethrins than the *T. confusum* populations. RR values obtained in bioassays suggest that insecticide resistance is not a widespread problem in Italian strains of *T. castaneum* and *T. confusum*, but there are populations in which the phenomenon exists. An extended monitoring activity could, therefore, be crucially important in adopting proper control measures for pest management.

Key words: contact insecticides, red flour beetle, confused flour beetle, Italy.

Introduction

Tribolium Macleay spp. is included in the list of the most important pests of cereal warehouses, silos and mills in Italy (Trematerra and Gentile, 2006; Trematerra and Süss, 2006), but causes damage throughout the world in a wide range of stored products (Rees, 1996). The two most common species, *Tribolium castaneum* (Herbst) and *Tribolium confusum* du Val, are important pests also in Italian pasta factories, where they are carried with raw materials (Trematerra and Süss, 2006).

Despite an increasing interest in Europe in a potential application of biological control to stored-product pests (Hansen, 2007), insect pest control in Italian warehouses and stocking facilities, as well as worldwide, is still based on the use of fumigants and contact insecticides (Donahaye, 2000, Trematerra and Gentile, 2006). As a consequence of repeated treatments with chemical insecticides, many cases of insecticide resistance have been detected in the genus *Tribolium* around world. The first records date back to the end of 1959s and the 1st half of 1960s (Anonymous, 1958; Kumar and Morrison, 1965). Cases of resistance are known all over the world and refer to the main classes of insecticides used in stored products such as phosphine, methyl bromide, organophosphates, pyrethroids and insect growth regulators (Anisur-Rahman and Shahjahan, 2000; Champ and Dyte, 1976; Collins, 1998; Dhaliwal and Chawla, 1995; El-Lakwah *et al*., 1996; Horowitz *et al*., 1998; Pacheco *et al*., 1994; Pimentel *et al*., 2007; Werner, 1997; Zettler and Arthur, 1997). In some cases, the effects of the selective pressure on *Tribolium* populations resulted in a better ecological fitness compared to the susceptible strains (enhanced tolerance to temperature and humidity stresses) (Shukla *et al*., 1989; Williams, 1989), or in reproductive performances that were better in malathion resistant males than in susceptible ones (Arnaud and Haubruge, 2002).

In Italy, the spread of insecticide resistance in *Tribolium* spp. populations is not extensively investigated. In this paper the authors give a further extent of a preliminary baseline data (Rofrano *et al*., 2009) on response to contact insecticides in *T. castaneum* and *T. confusum* populations collected in storage commodities in Italy.

Materials and methods

Populations and rearing conditions

Eighteen populations, seven of *T. castaneum* and eleven of *T. confusum* were collected in different Italian sites, distributed along the peninsula and in Sicily. Identification of species was carried out according to Rees (2004). As shown in table 1, adults and larvae of these populations were collected in warehouses; the susceptible populations were IEM-ca and IEM-co which had been reared in the lab for at least five years without any insecticide selection and were taken as the control for *T. castaneum* and *T. confusum*, respectively. The Catania population derived from adults and larvae collected in at least two different Sicilian sites. Each population was reared inside plastic box (20 x 25 x 15 cm) containing wheat flour covered by a lid with holes for ventilation. Every 7 days, the adults were removed and transferred to another box with new substrate. In this way, adults obtained in the following generations from each box were of similar age (7 ± 2 days). These adults were used in bioassays.

Table 1. Populations of *T. castaneum* and *T. confusum* used in bioassays.

Population name	Locality	Region	Storage facility
T. castaneum			
IEM-ca	Milano	Lombardy	Lab rearing
Ente Risi	Mortara	Lombardy	Rice mill
Ferrara	Ferrara	Emilia-Romagna	Mill
Vitillo	Benevento	Campania	Grain silos
T_ca_27	Busso	Molise	Grain warehouse
T_ca_43	Fossato	Molise	Grain warehouse
T_ca_44	Altamura	Apulie	Grain warehouse
T_ca_45	Altamura	Apulie	Grain warehouse
T. confusum			
IEM-co	Milano	Lombardy	Lab rearing
T_co_003	Hinterland Milano	Lombardy	Grain silos
Catania	Catania	Sicily	Unknown
T_co_23	S. Bartolomeo	Campania	Bakery
T_co_26	Busso	Molise	Grain warehouse
T_co_28	Fossato	Molise	Grain warehouse
T_co_29	Nola	Campania	Bakery
T_co_34	S. Bartolomeo	Campania	Bakery
T_co_35	Campobasso	Molise	Unknown
T_co_36	S. Bartolomeo	Campania	Grain warehouse
T_co_42	S. Bartolomeo	Campania	Grain warehouse
T_co_48	Fossato	Molise	Silos

Insecticides

Six technical grade insecticides were used in bioassays: deltamethrin, malathion, cypermethrin, pyrethrins, diazinon and chlorpyrifos; all were supplied by Blueline laboratories (Forlì, Italy), with the exception of chlorpyrifos, supplied by Sigma Aldrich. Some of these chemicals were chosen among the most common insecticides used for chemical control of insect pests in Italian food storage facilities or directly on grain (deltamethrin, malathion and pyrethrins), or in warehouses (diazinon, cypermethrin and chlorpyrifos) (Süss, personal communication; Domenichini, 2001).

Bioassays

Insecticides were applied by topical applications. Bioassays were carried out using a Burkard microapplicator with a 100 µl syringe; 1 µl of solution of technical grade insecticide in technical grade acetone was applied on the adult thorax. The doses assayed produced mortality ranging from 2 to 98%. A control treated with 1 µl of acetone was run in each test. At least four replicates of 10 unsexed adults were assayed for insecticide. Treated adults were placed respectively in Petri dishes with the bottom covered by a filter paper disk and held inside a rearing chamber at 25 ± 1 °C and 60 ± 5% RH. Mortality was checked after 48 h.

Statistical analysis

Mortality data from insecticide treated strains were corrected for control mortality by Abbott's formula (Abbott, 1925), transformed in logits and analysed by POLO-PC (LeOra Software, 1987). A population was considered to be significantly more (or less) resistant than another when there was no overlapping of 95% CL of the LD_{50}. Relative susceptibility of populations to chemicals was estimated by resistance ratios (RR = LD_{50} field strain / LD_{50} susceptible strain).

Principal Component Analysis (PCA) was used to reveal the differences among *Tribolium* populations on the basis of their susceptibility to the tested insecticides. A data matrix was produced with RR values calculated for each population and each insecticide. A software package (Primer-E Ltd., 2006) was used to perform PCA.

Results

The main parameters of LD-lines obtained for each tested insecticide in *T. castaneum* and *T. confusum* populations are shown in tables 2 to 7.

Deltamethrin

In *T. castaneum* bioassays, the slopes ranged between a minimum of 1.84 (for T_ca_27 population) and a maximum of 3.22 (for T_ca_44 population) and LD_{50} values ranged from the values of 1 x 10^{-5} of the Ente Risi population to 2.8 x 10^{-4} of T_ca_43 population. The highest RR values were observed in T_ca_27 (RR = 7), T_ca_43 (RR = 14), T_ca_44 (RR = 8) and T_ca_45 (RR = 6) populations, unless only T_ca_43 and T_ca_44 populations were significantly more resistant than the IEM-ca population (table 2).

Bioassays on *T. confusum* generally showed the lowest slope values (slope values ranging from 1.31 for T_co_42 population to 2.95 for T_co_35 population) and LD_{50}s included in the interval between 2 x 10^{-5} in T_co_003 population and 1 x 10^{-3} in T_co_48 population, where the highest RR value was recorded (RR = 16.7) (table 2).

Table 2. Dose-mortality response of *Tribolium* spp. populations to deltamethrin.

Population name	Slope ± S.E	LD_{50} (ppm)	95% CL[1]		χ^2 (df)	RR[2]
T. castaneum						
IEM-ca	2.62 ± 0.62	2x10^{-5}	0.7x10^{-5}-4.5x10^{-5}	a	0.105 (2)	-
Ente Risi	2.03 ± 0.61	1x10^{-5}	-	-	3.769 (2)	0.5
Ferrara	2.55 ± 0.69	3x10^{-5}	-	-	2.760 (2)	1.5
Vitillo	2.66 ± 0.43	3x10^{-5}	2x10^{-5}–4x10^{-5}	a	0.350 (2)	1.5
T_ca_27	1.84 ± 0.54	1.4x10^{-4}	2.6x10^{-5}–2.8x10^{-4}	ab	0.219 (2)	7
T_ca_43	2.43 ± 0.58	2.8x10^{-4}	1.3x10^{-4}–4.7x10^{-4}	b	0.015 (2)	14
T_ca_44	3.22 ± 0.83	1.6x10^{-4}	7.5x10^{-5}–2.6x10^{-4}	b	0.284 (1)	8
T_ca_45	2.20 ± 0.61	1.2x10^{-4}	2.9x10^{-5}–2.3x10^{-4}	ab	1.484 (2)	6
T. confusum						
IEM-co	1.88 ± 0.48	6x10^{-5}	3x10^{-5}–1.1x10^{-4}	ab	0.161 (2)	-
Catania	2.03 ± 0.51	3x10^{-5}	1x10^{-5}–4x10^{-5}	a	5.180 (3)	0.5
T_co_003	2.26 ± 0.60	2x10^{-5}	1x10^{-5}–3x10^{-5}	a	0.311(2)	0.3
T_co_23	2.02 ± 0.53	2.3x10^{-4}	8.2x10^{-5}–4.3x10^{-4}	bc	0.505 (2)	3.8
T_co_26	1.51 ± 0.41	9.8x10^{-5}	1.5x10^{-5}–2.1x10^{-4}	ab	0.177 (2)	1.6
T_co_28	1.73 ± 0.47	4.3x10^{-4}	1.7x10^{-4}–8.5x10^{-4}	bc	0.286 (2)	7.2
T_co_29	2.60 ± 0.76	1.6x10^{-4}	1x10^{-5}–3.9x10^{-4}	abc	1.690 (1)	2.7
T_co_34	1.72 ± 0.52	7.8x10^{-5}	0.9x10^{-5}–1.7x10^{-4}	ab	0.431 (2)	1.3
T_co_35	2.95 ± 0.77	2x10^{-4}	9.1x10^{-5}–3.2x10^{-4}	bc	0.059 (1)	3.3
T_co_36	1.73 ± 0.38	5.7x10^{-4}	3x10^{-4}–9.9x10^{-4}	cd	1.690 (2)	9.5
T_co_42	1.31 ± 0.45	1.6x10^{-4}	1x10^{-5}–3.9x10^{-4}	abc	0.396 (2)	2.7
T_co_48	1.50 ± 0.44	1x10^{-3}	4.6x10^{-4}–2.9x10^{-3}	d	0.518 (2)	16.7

[1] Different letters indicate non-overlap of confidence limits ($P < 0.05$).
[2] Resistance ratio = LD_{50} resistant population / LD_{50} susceptible population.

Cypermethrin

Bioassays on *T. castaneum* with cypermethrin gave the results shown in table 3. The slopes of LD-lines ranged from 0.76 (Ente Risi population) to 2.59 (T_ca_43 population) and LD_{50}s were included in the interval 2 x 10^{-5} (susceptible population, IEM-*ca*) and 3 x 10^{-4} (T_ca_44 population); the highest RR values were detected in Ferrara, T_ca_44 and T_ca_45 populations, (RR = 8, 14 and 8 respectively). However, in Ferrara population the linearity for dose mortality response was rejected ($P < 0.05$).

In *T. confusum*, the slopes varied between 1.4 (Catania population) and 2.6 (T_co_34 population), LD_{50}s ranged from 4 x 10^{-5} (Catania population) and 3.2 x 10^{-4} (T_co_48 population), while RR values found for *T. confusum* populations were low (0.3 < RR < 2.7). In this batch of bioassays, the linearity for dose mortality response was rejected for the susceptible strain IEM-ca ($P < 0.05$).

Malathion

In both species no resistance to malathion was observed, so that RR values were low and were between 0.1 (T_ca_43, T_ca_44 and T_ca_45 populations of *T. castaneum*) and 3.4 (T_co_36 population of *T. confusum*) (see table 4). In bioassays on *T. castaneum*, LD_{50}s of Ente Risi, Vitillo and T_ca_43 populations were significantly more susceptible than IEM-ca strain.

Linearity of dose mortality response was rejected for IEM-ca, Ferrara, Vitillo, T_ca_44 and T_ca_45 in *T. castaneum* populations and for Catania population in *T. confusum*.

Diazinon

As shown in table 5, bioassays on *T. castaneum* did not show any resistance level in all the populations tested (RR values ranging from 0.5 to 3), while in *T. confusum* populations, RR calculated ranged from 0.5 (T_co_003 population) to 10.4 (T_co_36 population), with the slopes varying between 0.49 (T_co_35 population) and 3.59 (IEM-co population). However, linearity of dose mortality response was rejected in one population of *T. castaneum* (Vitillo) and in two of *T. confusum* (T_co_34 and T_co_42).

Chlorpyrifos

Table 6 shows main parameters of LD-lines for chlorpyrifos. *T. castaneum* bioassays showed the highest RR value in the Ferrara population (RR = 11.7) with a slope of 2.77; in *T. confusum*, an RR of 9.3 was observed in the T_co_29 population. Data set of Vitillo population in *T. castaneum* and T_co_36 in *T. confusum* did not fit the logit model ($P < 0.05$).

Pyrethrins

Results obtained in bioassays with pyrethrins are shown in table 7. Linearity for dose mortality response was rejected for T_ca_44 and T_ca_45 populations in *T. castaneum* and for T_co_28, T_co_42 and T_co_48 in *T. confusum*. The highest RR values were observed in the T_ca_27 population for *T. castaneum* (RR = 4.9) and in T_co_36 population for *T. confusum* (RR = 8.6).

Table 3. Dose-mortality response of *Tribolium* spp. populations to cypermethrin.

Population name	Slope ± S.E	LD_{50} (ppm)	95% CL[1]		χ^2 (df)	RR[2]
T. castaneum						
IEM-ca	1.34 ± 0.48	$2x10^{-5}$	$8.8x10^{-6}$–$4.2x10^{-5}$	a	4.848 (2)	-
Ente Risi	0.76 ± 0.45	$6x10^{-5}$	$4.2x10^{-5}$–$1.1x10^{-4}$	ab	0.136 (2)	3
Ferrara	1.85 ± 0.38	$1.6x10^{-4}$	$6.6x10^{-5}$–$2.7x10^{-4}$	b	12.492 (3)*	8
Vitillo	1.18 ± 0.32	$6x10^{-5}$	$4.2x10^{-5}$–$1.1x10^{-4}$	ab	6.690 (4)	3
T_ca_27 not tested						
T_ca_43	2.59 ± 0.64	$1.2x10^{-4}$	$8.4x10^{-5}$– $3.3x10^{-4}$	b	0.773 (2)	6
T_ca_44	2.44 ± 0.47	$3x10^{-4}$	$1.7x10^{-4}$– $4.6x10^{-4}$	b	0.892 (2)	14
T_ca_45	2.11 ± 0.46	$1.6x10^{-4}$	$6.6x10^{-5}$– $2.7x10^{-4}$	b	0.075 (2)	8
T. confusum						
IEM-co	2.3 ± 0.42	$1.2x10^{-4}$	$5x10^{-5}$–$4.2x10^{-4}$	ab	6.021 (2)*	-
Catania	1.4 ± 0.43	$4x10^{-5}$	$1x10^{-5}$–$1x10^{-4}$	a	4.152 (2)	0.3
T_co_003	2.21 ± 0.66	$1.7x10^{-4}$	$9x10^{-5}$–$3.2x10^{-4}$	ab	1.758 (2)	1.4
T_co_23	1.70 ± 0.51	$1.5x10^{-4}$	$2.7x10^{-5}$–$3.2x10^{-4}$	ab	0.490 (2)	1.2
T_co_26	2.34 ± 0.49	$1.7x10^{-4}$	$8.2x10^{-5}$–$2.8x10^{-4}$	ab	0.057 (2)	1.4
T_co_28	2.46 ± 0.52	$1.9x10^{-4}$	$9.7x10^{-5}$–$3x10^{-4}$	ab	0.989 (2)	1.6
T_co_29 not tested						
T_co_34	2.60 ± 0.60	$2.1x10^{-4}$	$9.4x10^{-5}$–$3.3x10^{-4}$	ab	1.030 (2)	1.7
T_co_35 not tested						
T_co_36	2.54 ± 0.49	$2.8x10^{-4}$	$1.6x10^{-4}$–$4.2x10^{-4}$	b	0.697 (2)	2.3
T_co_42	2.15 ± 0.55	$2.1x10^{-4}$	$7.3x10^{-5}$–$3.7x10^{-4}$	ab	0.387 (2)	1.7
T_co_48	2.30± 0.56	$3.2x10^{-4}$	$1.5x10^{-4}$–$5.5x10^{-4}$	b	0.066 (2)	2.7

[1] Different letters indicate non-overlap of confidence limits ($P < 0.05$).
[2] Resistance ratio = LD_{50} resistant population / LD_{50} susceptible population.
* χ^2 testing linearity of dose-mortality response ($P < 0.05$).

Table 4. Dose-mortality response of *Tribolium* spp. populations to malathion.

Population name	Slope ± S.E	LD_{50} (ppm)	95% CL[1]		χ^2 (df)	RR[2]
T. castaneum						
IEM-ca	1.89 ± 0.32	0.012	$7.5x10^{-3}$–0.015	a	12.855 (4)*	-
Ente Risi	2.43 ± 0.35	$2.36x10^{-3}$	$1.45x10^{-3}$–$3.82x10^{-3}$	b	2.599 (2)	0.2
Ferrara	2.28 ± 0.30	$1.83x10^{-3}$	-	b	20.307 (4)*	0.2
Vitillo	2.19 ± 0.23	$3.37x10^{-3}$	$1.94x10^{-3}$–$5.54x10^{-3}$	b	28.660 (4)*	0.3
T_ca_27 not tested						
T_ca_43	1.62 ± 0.45	$8.4x10^{-4}$	$3.8x10^{-4}$–$2x10^{-3}$	b	0.401 (2)	0.1
T_ca_44	3.74 ± 0.71	$7.3x10^{-4}$	-	-	9.218 (2)*	0.1
T_ca_45	2.40 ± 0.46	$1.2x10^{-3}$	-	-	6.185 (2)*	0.1
T. confusum						
IEM-co	3.72 ± 0.74	$2.1x10^{-4}$	$9x10^{-5}$–$2.6x10^{-4}$	a	9.905 (5)	-
Catania	1.86 ± 0.60	$4.9x10^{-4}$	$2.9x10^{-4}$–$1.2x10^{-3}$	b	6.250 (2)*	2.3
T_co_003	2.42 ± 0.63	$1.4x10^{-4}$	$6x10^{-5}$–$2.2x10^{-4}$	a	0.338 (4)	0.6
T_co_23 not tested						
T_co_26	1.85 ± 0.44	$1.3x10^{-4}$	$4.2x10^{-5}$–$2.4x10^{-4}$	a	0.566 (2)	0.6
T_co_28	1.78 ± 0.34	$5.2x10^{-4}$	$3x10^{-4}$–$7.9x10^{-4}$	b	1.309 (2)	2.5
T_co_29	0.84 ± 0.55	$2.8x10^{-4}$	-	-	2.309 (1)	1.3
T_co_34	2.32 ± 0.58	$8x10^{-5}$	$2.1x10^{-5}$–$1.4x10^{-4}$	a	1.069 (2)	0.4
T_co_35	6.11 ± 1.29	$2.2x10^{-4}$	-	-	1.879 (1)	1
T_co_36	3.31 ± 0.61	$7.1x10^{-4}$	$5x10^{-4}$–$1x10^{-3}$	b	0.814 (2)	3.4
T_co_42	4.26 ± 0.83	$5.3x10^{-4}$	$3.8x10^{-4}$–$7x10^{-4}$	b	0.928 (2)	2.5
T_co_48	2.79 ± 0.52	$4.4x10^{-4}$	$2.8x10^{-4}$–$6.4x10^{-4}$	b	0.501 (2)	2.1

[1] Different letters indicate non-overlap of confidence limits ($P < 0.05$).
[2] Resistance ratio = LD_{50} resistant population /LD_{50} susceptible population.
* χ^2 testing linearity of dose-mortality response ($P < 0.05$).

Table 5. Dose-mortality response of *Tribolium* spp. populations to diazinon.

Population name	Slope ± S.E	LD_{50} (ppm)	95% CL[1]		χ^2 (df)	RR[2]
T. castaneum						
IEM-ca	3.49 ± 0.56	$4.3x10^{-4}$	$2.3x10^{-4}$ – $7.9x10^{-4}$	a	1.964 (3)	-
Ente Risi	3.04 ± 0.55	$2.7x10^{-4}$	$7.1x10^{-5}$–$4.8x10^{-4}$	a	4.612 (3)	0.6
Ferrara	1.99 ± 0.22	$1.32x10^{-3}$	$4.7x10^{-4}$–$3.3x10^{-3}$	a	2.309 (2)	3
Vitillo	3.88 ± 0.68	$5.1x10^{-4}$	$2.6x10^{-4}$–$8.1x10^{-4}$	a	23.747 (2)*	1.2
T_ca_27	2.12 ± 0.54	$2.2x10^{-4}$	$8x10^{-5}$–$4x10^{-4}$	a	0.948 (2)	0.5
T_ca_43	2.88 ± 0.54	$2.4x10^{-4}$	$6.9x10^{-5}$–$4.8x10^{-4}$	a	2.647 (2)	0.6
T_ca_44	2.99 ± 0.55	$2.8x10^{-4}$	-	-	3.971 (2)	0.6
T_ca_45	4.80 ± 0.99	$7.1x10^{-4}$	-	-	3.397 (2)	1.6
T. confusum						
IEM-co	3.59 ± 0.60	$2.5x10^{-4}$	$1.8x10^{-4}$–$3.3x10^{-4}$	a	2.280 (4)	-
Catania	2.34 ± 0.56	$5.6x10^{-4}$	$2.9x10^{-4}$–$8.7x10^{-4}$	abc	0.706 (3)	2.2
T_co_003	0.97 ± 0.74	$1.3x10^{-4}$	-	-	5.230 (3)	0.5
T_co_23	2.91 ± 0.66	$3.7x10^{-4}$	$2x10^{-4}$–$5.8x10^{-4}$	ab	0.189 (2)	1.5
T_co_26	1.91 ± 0.49	$4.8x10^{-4}$	$2.1x10^{-4}$– $9x10^{-4}$	abc	0.544 (2)	1.9
T_co_28	1.21 ± 0.26	$9.1x10^{-4}$	$4.3x10^{-4}$–$1.7x10^{-3}$	bcd	1.054 (3)	3.6
T_co_29	2.61 ± 0.48	$1.6x10^{-3}$	$9.8x10^{-4}$–$2.6x10^{-3}$	de	0.691 (3)	6.4
T_co_34	1.78 ± 0.36	$4.6x10^{-4}$	-	-	18.831 (2)*	1.8
T_co_35	0.49 ± 0.45	$8.9x10^{-4}$	$4.2x10^{-4}$–$1.6x10^{-3}$	bcd	4.302 (2)	3.6
T_co_36	3.21 ± 0.46	$2.6x10^{-3}$	$1.8x10^{-3}$–$3.7x10^{-3}$	e	1.443 (3)	10.4
T_co_42	2.63 ± 0.46	$2.1x10^{-3}$	$1.7x10^{-3}$–$3.5x10^{-3}$	de	7.991 (2)*	8.4
T_co_48	2.75 ± 0.51	$1x10^{-3}$	$6.8x10^{-4}$–$1.5x10^{-3}$	cd	1.328 (2)	4

[1] Different letters indicate non-overlap of confidence limits ($P < 0.05$).
[2] Resistance ratio = LD_{50} resistant population / LD_{50} susceptible population.
* χ^2 testing linearity of dose-mortality response ($P < 0.05$).

Table 6. Dose-mortality response of *Tribolium* spp. populations to chlorpyrifos.

Population name	Slope ± S.E	LD_{50} (ppm)	95% CL[1]		χ^2 (df)	RR[2]
T. castaneum						
IEM-ca	1.69 ± 0.59	$4x10^{-5}$	0 –$9x10^{-5}$	a	1.758 (1)	-
Ente Risi	2.25 ± 0.57	$1.1x10^{-4}$	$4.4x10^{-5}$–$1.9x10^{-4}$	a	2.952 (2)	2.7
Ferrara	2.77 ± 0.78	$4.7x10^{-4}$	$2.7x10^{-4}$–$7.3x10^{-3}$	b	2.571 (2)	11.7
Vitillo	1.75 ± 0.37	$1.6x10^{-4}$	$1.1x10^{-4}$–$2.3x10^{-4}$	a	11.829 (4)*	4
T_ca_27	3.28 ± 0.93	$1.1x10^{-4}$	$4.4x10^{-5}$–$1.9x10^{-4}$	a	0.037 (1)	2.7
T_ca_43	3.19 ± 0.61	$1.7x10^{-4}$	$4.4x10^{-5}$–$3.3x10^{-4}$	ab	2.641 (2)	4.2
T_ca_44	2.36 ± 0.74	$6.1x10^{-5}$	$7.3x10^{-6}$–$1.2x10^{-4}$	a	0.540 (1)	1.5
T_ca_45	4.72 ± 0.86	$1.8x10^{-4}$	$1.2x10^{-4}$–$2.5x10^{-4}$	a	0.046 (1)	4.5
T. confusum						
IEM-co	1.78 ± 0.49	$6x10^{-5}$	$1x10^{-5}$–$1.1x10^{-4}$	a	2.431 (3)	-
Catania	2.18 ± 0.51	$3.4x10^{-4}$	$1.9x10^{-4}$–$6.9x10^{-4}$	b	0.872 (2)	5.6
T_co_003	1.68 ± 0.48	$5x10^{-5}$	$1x10^{-5}$–$1.1x10^{-4}$	a	0.478 (2)	0.8
T_co_23	2.83 ± 0.65	$2.8x10^{-4}$	$1.4x10^{-4}$–$4.4x10^{-4}$	b	0.550 (2)	4.7
T_co_26	2.13 ± 0.49	$1.2x10^{-4}$	$4.1x10^{-5}$–$2.1x10^{-4}$	ab	0.258 (2)	2
T_co_28	3.07 ± 0.56	$4.1x10^{-4}$	$2.7x10^{-4}$–$5.8x10^{-4}$	b	0.001 (2)	6.8
T_co_29	3.78 ± 0.71	$5.6x10^{-4}$	$4x10^{-4}$–$7.7x10^{-4}$	b	0.768 (2)	9.3
T_co_34	1.50 ± 0.46	$4.5x10^{-5}$	$1x10^{-5}$–$1x10^{-4}$	a	2.671 (2)	0.7
T_co_35	2.19 ± 0.57	$1.9x10^{-4}$	$6.6x10^{-5}$–$3.4x10^{-4}$	ab	0.109 (2)	3.2
T_co_36	3.59 ± 0.66	$4.8x10^{-4}$	$3.3x10^{-4}$–$6.2x10^{-4}$	b	4.395 (2)*	8
T_co_42	3.09 ± 0.57	$5.5x10^{-4}$	$3.7x10^{-4}$–$7.9x10^{-4}$	b	1.591 (2)	9.2
T_co_48	1.14 ± 0.40	$4.5x10^{-5}$	$2x10^{-6}$–$1.4x10^{-4}$	a	1.117 (2)	0.7

[1] Different letters indicate non-overlap of confidence limits ($P < 0.05$).
[2] Resistance ratio = LD_{50} resistant population / LD_{50} susceptible population.
* χ^2 testing linearity of dose-mortality response ($P < 0.05$).

Table 7. Dose-mortality response of *Tribolium* spp. populations to pyrethrins.

Population name	Slope ± S.E	LD_{50} (ppm)	95% CL[1]		χ^2 (df)	RR[2]
T. castaneum						
IEM-ca	2.09 ± 0.48	$4.9x10^{-4}$	$2.8x10^{-4}$–$8.5x10^{-4}$	a	1.271 (3)	-
Ente Risi	2.37 ± 0.66	$5.8x10^{-4}$	$3x10^{-4}$–$9.8x10^{-4}$	a	0.029 (2)	1.2
Ferrara	1.88 ± 0.41	$7.3x10^{-4}$	$2.9x10^{-4}$–$1.4 x10^{-3}$	ab	1.277 (2)	1.5
Vitillo	3.18 ± 0.52	$1.0x10^{-3}$	$7.4x10^{-4}$–$1.5 x10^{-3}$	ab	1.220 (3)	2.1
T_ca_27	1.88 ± 0.62	$2.4x10^{-3}$	$1x10^{-3}$–$2.2x10^{-2}$	b	0.452 (2)	4.9
T_ca_43	1.83 ± 0.39	$6.1x10^{-4}$	$2x10^{-4}$–$1.3x10^{-3}$	ab	3.537 (3)	1.2
T_ca_44	2.30 ± 0.42	$1.5x10^{-3}$	$9.5x10^{-4}$–$1.9 x10^{-3}$	ab	8.809 (2)*	3.1
T_ca_45	2.66 ± 0.43	$9.1x10^{-4}$	$3.1x10^{-4}$–$2.5x10^{-3}$	ab	9.022 (3)*	1.9
T. confusum						
IEM-co	2.50 ± 0.55	$3.7x10^{-4}$	$2.3x10^{-4}$–$5.9x10^{-4}$		2.319 (3)	-
Catania	2.20 ± 0.58	$1.1x10^{-3}$	$6.4x10^{-4}$–$2.2x10^{-3}$		1.111 (2)	3
T_co_003	1.93 ± 0.60	$5.2x10^{-4}$	$2x10^{-4}$–$9.5x10^{-4}$		0.099 (2)	1.4
T_co_23	2.09 ± 0.40	$1.6x10^{-3}$	$6x10^{-4}$–$3.7x10^{-3}$		3.843 (2)	4.3
T_co_26	1.56 ± 0.45	$3.4x10^{-4}$	$1x10^{-4}$–$7.2x10^{-4}$		0.095 (2)	0.9
T_co_28	2.12 ± 0.41	$1.8x10^{-3}$	$6.3x10^{-4}$–$3.9x10^{-3}$		7.456 (2)*	4.9
T_co_29	1.56 ± 0.31	$7.5x10^{-4}$	$3.9x10^{-4}$–$1.3x10^{-3}$		0.840 (3)	2
T_co_34	1.98 ± 0.38	$7.1x10^{-4}$	$2.3x10^{-4}$–$1.8x10^{-3}$		2.788 (2)	1.9
T_co_35	2.06 ± 0.41	$1x10^{-3}$	$5.5x10^{-4}$–$1.8x10^{-3}$		1.523 (3)	2.7
T_co_36	2.02 ± 0.42	$3.2x10^{-3}$	$2x10^{-3}$–$6.8x10^{-3}$		1.120 (2)	8.6
T_co_42	2.33 ± 0.39	$1.9x10^{-3}$	$7.4x10^{-4}$–$4x10^{-3}$		9.965 (3)*	5.1
T_co_48	2.53 ± 0.37	$1.5x10^{-3}$	$5.9x10^{-4}$–$3.6x10^{-3}$		8.911 (3)*	4.1

[1] Different letters indicate non-overlap of confidence limits ($P < 0.05$).
[2] Resistance ratio = LD_{50} resistant population / LD_{50} susceptible population.
* χ^2 testing linearity of dose-mortality response ($P < 0.05$).

Table 8. Coefficients in the linear combinations of variables making up PC's.

Variable	PC1	PC2	PC3	PC4	PC5
Deltamethrin	0.130	0.712	0.072	–0.210	0.645
Cypermethrin	–0.348	0.502	0.433	–0.056	–0.475
Malathion	0.587	–0.075	–0.128	0.048	0.124
Diazinon	0.528	–0.012	0.210	–0.681	–0.381
Chlorpyrifos	0.081	–0.350	0.861	0.158	0.324
Pyrethrins	0.481	0.336	0.077	0.680	–0.305

Using the statistical method previously adopted by other authors (Pap and Farkas, 1994), PCA was applied to RR values in both species: the results of the analysis are shown in figure 1. PC1 and PC2 were the most important components, accounting for 72% of variance (45.1% and 26.9%, respectively), so that a two dimensional graph gives a good description of the variation in population resistance. Along the PC1 axis, a clear-cut separation in two groups of *Tribolium* populations can be observed, with a gap around the 0 value, according to the species. All populations of *T. castaneum* and three of *T. confusum* were placed in the left part of the graph, while the remaining populations of *T. confusum* were situated in the opposite side, where malathion, diazinon and pyrethrins showed the main weight in the linear combination of insecticide resistance variables. On the other hand, there was no gap along PC2, where the two species have a rather homogeneous distribution, without any cluster of experimental points. However, many tested populations (14 out of the 18) are positioned in the upper half of the graph, where the main contribution is given by the vectors of deltamethrin, cypermethrin and pyrethrins, respectively (table 8 and figure1).

Discussion and conclusions

The occurrence of resistance to insecticides in *Tribolium* populations is a worldwide problem (Champ and Dyte, 1976; Arthur, 1996): resistance cases have been detected in America (Halliday *et al*., 1988; Haliscak and Beeman, 1983, Stadler *et al*., 2003), as well as in Asia (Saxena *et al*., 1991a; 1991b; Sinha and Saxena, 2001) and in Australia (Collins, 1998). Comparison of data is not easy because of the different bioassay protocols used and for the huge variety of insecticides used.

The few data available date back to the end of 1980s , with two resistance cases detected to lindane and malathion in *T. castaneum* and *T. confusum* strains imported from abroad (Contessi, 1989). Recently resistance to phosphine was detected in some field popula-

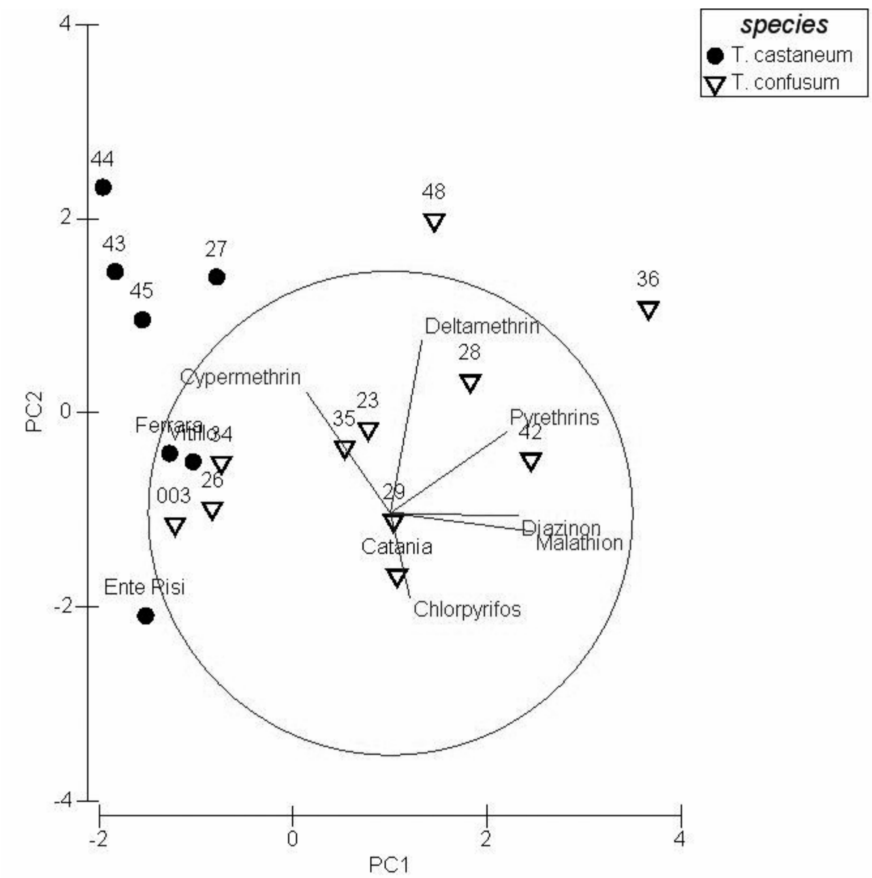

Figure 1. PCA of treated *T. castaneum* and *T. confusum* populations, where PC1 and PC2 accounted for 45.1% and 26.9% of the variance in the data, respectively.

tions of *Tribolium* spp. (Savoldelli and Süss, 2008). However, the assessment of pesticide resistance in Italian *Tribolium* populations as well as extensive insecticide resistance monitoring would be critical in achieving a rational application of IPM strategies in stored products, especially because of the currently massive import of foodstuffs (Savoldelli and Süss, 2008). A previous paper (Rofrano *et al.* 2009) gave a preliminary contribution to fill this gap.

As far as our results are concerned, we observed insecticide resistance in Italian populations of the red flour beetle and of the confused flour beetle with resistance detected more frequently in T. *castaneum* than in *T. confusum*. Although no full records of insecticide treatments were available for the storage areas where the samples were collected data obtained in bioassays showed that the highest resistance levels were observed to the pyrethroids deltamethrin and cypermethrin. This is consistent with the available information about the use of insecticides in Italian storage food facilities (Domenichini, 2001; Trematerra and Gentile, 2006) according to which the pyrethroids (deltamethrin in particular) are commonly used in grain treatment. Lowest resistance levels were observed in bioassays with the oldest insecticides tested (diazinon, malathion and pyrethrins), reasonably because these pesticides are not used anymore.

PCA is consistent with the obtained results, showing as 14 populations of the 18 bioassayed lie in the part of the graph where deltamethrin, cypermethrin and pyrethrins give the main positive contribution to resistance. The distribution of data along PC1 axis indicates low levels of resistance to malathion and diazinon in both species and in particular, in *T. castaneum* whose populations are completely located in the left part of the graph, divided by a sharp gap by the *T. confusum* populations. This separation could mean a different susceptibility of the two species compared to malathion and diazinon.

PCA showed a good potential as a tool in wide screening of insecticide resistance at population level, because it allows to have a broad and simultaneous view of the influence exerted by the different insecticides tested.

The resistance level observed suggests that the insecticide resistance phenomenon is widespread, although not dramatic, and cannot be neglected when control measures are undertaken. Moreover, in Italy grain imports are increasing, so that resistant populations of *Tribolium* spp. could be introduced at any time: this fact strengthens the importance of extensive monitoring of resistance, with a view to rational rotation of insecticides in order to delay its onset.

Acknowledgements

We thank our colleagues F. Chiesa, A. Russo, L. Süss, P. Trematerra, and their collaborators for the precious help in strain collection. This work was supported by a grant of the M.I.U.R.

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Received February 9, 2010. Accepted October 8, 2010.