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RESPONSE OF STORED PRODUCT PEST LIFE STRATEGY TO CHEMICAL TREATMENT: EFFECT OF TRICALCIUM PHOSPHATE (TCP) ON *TRIBOLIUM* STRAINS (*T. CASTANEUM* HERBST AND *T. CONFUSUM* DUVAL)

ABSTRACT: The experiments were carried out on two strains: cI of *Tribolium castaneum* (Hbst.) and bIV of *T. confusum* Duval. in medium enriched by yeast using tricalcium phosphate (TCP) as the controling factor. Within these two species two groups of 6- and 7-instar individuals were discerned. The developmental time and survival of *T. confusum* population is depressed stronger than those of *T. castaneum*. There are no differences between 6- and 7-instar groups in response to various concentration of TCP. The results obtained for fecundity showed that this trait is more restricted in *T. castaneum* than in *T. confusum*. Hatchability in all experiments was similar.

KEY WORDS: stored product pest, chemical control survival, fecundity, hatchability, *Tribolium*

1. INTRODUCTION

In previous paper (P r u s 1996) the response to tricalcium phosphate (TCP) in *Tribolium* strain cultured in medium consisting of flour and different concentrations of TCP was studied. In this experiment medium in which the insects were culcured was enriched by yeast. In such medium (flour + yeast) all studies on these species were performed including populational, physiological, bioenergetical and genetical studies. It is known as standard medium in literature devoted to *Tribolium* studies.

Such conditions can be observed in stores or in shops when the flour is stored in places with high humidity. Low constant humidities, below 65% relative humidity or below 14% moisture content, are generally preferred because they inhibit the development of fungi. However, aridity usually has less influence on insects than it does on fungi. (E v a n s 1987).

It caused mass developing of fungi enriched with vitamins from B group which also caused increase of fecundity in stored product insect. (Park et al. 1961). To protect grain from stored product pests chemical method is commonly used. For such protection the pesticides such as juvenile hormone analogues and photostable pyrethroids are often in use (Graham-Bryce 1987). Another method applied is genetic control which based mainly on sterile insect techniques. This method can be applied when some requirements are met: the target species must be a key pest with substantial economic or public health impact; the ecology, biology and behaviour of the target species must be known in detail and sterilization must be possible as well as quality control procedures must be available for monitoring and possibly correcting deterioration of traits caused by inadequate rearing or handling procedure (Boller 1987). This method is commonly used for agricultural crop pests. The above mentioned methods are unusable for protection of flour, so in this case another methods are useful; such as inorganic salts inhibiting development of insect and fecuntity (e.g. TCP) (Boczek 1984) or liquid from special plants for example vapours of *Acorus calamus* L. oil (Risha et al. 1990) or new method petroleum ether extracted *Annona squamosa* Linn. seed oil for reduction of adult emergence of *T. castaneum* Hbst. (Parveen and Selman 1995).

The paper aimed at learning about response of 6- and 7-instar groups of two strains of *Tribolium* to different concentrations of tricalcium phosphate in food enriched by yeast.

2. MATERIAL AND METHODS

Experiments were carried out on two strains of *Tribolium*:cI of *T. castaneum* (Hbst) and bIV of *T. confusum* Duval, both originating from the University of Chicago (Park et al. 1961). In each strain 6- and 7-instar groups were discerned and cultured at 29°C and relative humidity of 75% in a dark incubator, Cultured medium was wheat flour and baker's yeast in weight proportion 20 : 1. To this medium TCP was added in adequate proportions to obtain three concentrations: 0.5, 1.0 and 1.5% (by weight). The substance originated from USDA Laboratory, Savanah, Georgia.

To 50 g of culture medium (control) and medium with various concentration of TCP a hundred newly laid eggs were placed. After the egg hatching, the number of larvae was recorded every two days. Later on the pupation and eclosion moments were ascertained with simultaneous record of reduction in numbers of pupae and adults. The whole set of experiments involved the following design: two species × two instar groups × four TCP concentrations × 3 replications which gives a total of 48 samples.

In experiments on fecundity the animals that succeeded in eclosion in the experiment described above were mated and kept in vials as single pairs in 8 g of either standard culture medium or with different concentrations of TCP. Animals from controls were put into control medium and those from different concentrations of TCP to the same TCP concentrations. The experiments were run for 30 days with counts of eggs done every 3 days. The eggs thus collected were transferred to small containers and left over in incubator for 6 days to de-

velop. Then hatchability of eggs was estimated as percentage of eggs that hatched.

Two additional series of experiments were performed where animals cultured initially at TCP concentration of 0.5 or 1.0% were transferred to standard culture medium in which their fecundity and hatchability were tested. This allowed to evaluate the effect of a temporary lack of harmful agent in the medium on parameters tested.

The data obtained for fecundity were statistically elaborated using three factor design of analysis of variance (Simpson et al. 1960).

3. RESULTS

The effect of TCP on *T. castaneum* (Hbst.) cI and *T. confusum* Duval bIV strains, separately for 6- and 7-instar groups, was presented in the form of survival curves for each concentration of the inhibitory salt. In *T. castaneum,* 6-instar group shows stronger reaction than 7-instar one (Fig. 1A).The effect of TCP concentration is differentiated, resulting in higher mortality with increasing concentration. The strongest effect was observed in 6-instar group at 1.5% concentration of TCP, where only a few individuals survived to the pupal stage and they eclosed later. A strong decrease was observed in duration of the egg stage (25–40%) which reflects the combined effect of hatchability and mortality due to TCP.

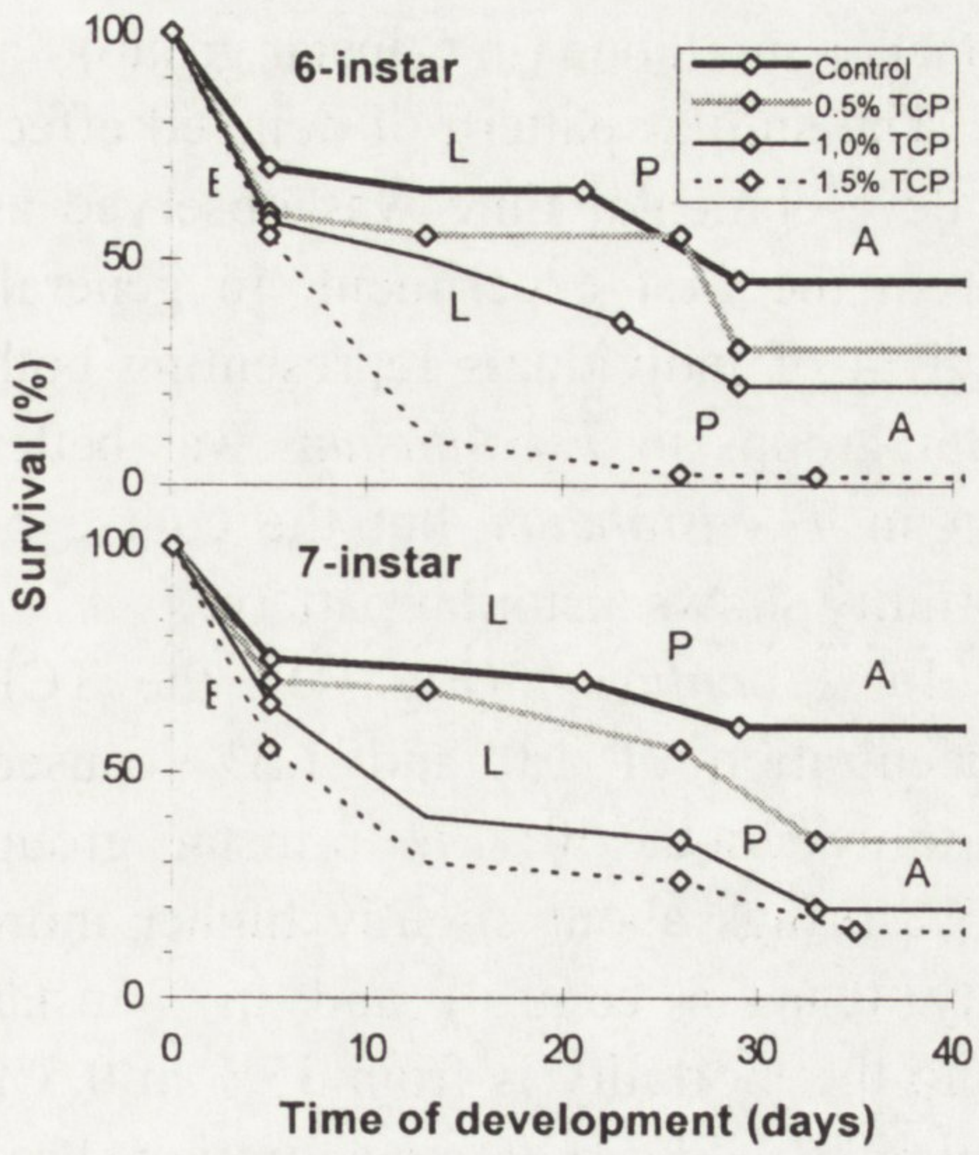

Fig. 1. Survival (%) from egg stage of *T. castaneum* 6-instar and 7-instar groups at various concentrations of tricalcium phosphate (TCP) E – eggs, L – larvae, P – pupae, A – adults

Further decrease follows in the early larval stages, with the curve reaching a certain plateau in elder larvae. Next rather strong decrease is observed in duration of pupal stage or at eclosion time. The total decrease in 6-instar group, as related to controls, amounts to 15% in 0.5%; 20 in 1.0% and over 40% in 1.5% of TCP concentrations. In 7-instar group corresponding percentages are: 25, 40 and 45%.

The development is prolonged at medium with TCP by about 5 days, except for concentration 1.0% in 6-instar group, where the shift in appearance of pupae amounts to two days as compared with the control.

In *T. confusum*, 6 instar group shows also stronger reaction to TCP than 7-instar group, although the effect is less differentiated (Fig. 2). In 6-instar group, a very low hatchability of eggs (55% of control) as compared with that in 7-instar group (70%) is observed. From these stage on, the survival in controls of 6-instar group is rather good (only 10% decrease) and mortality due to the salt effect

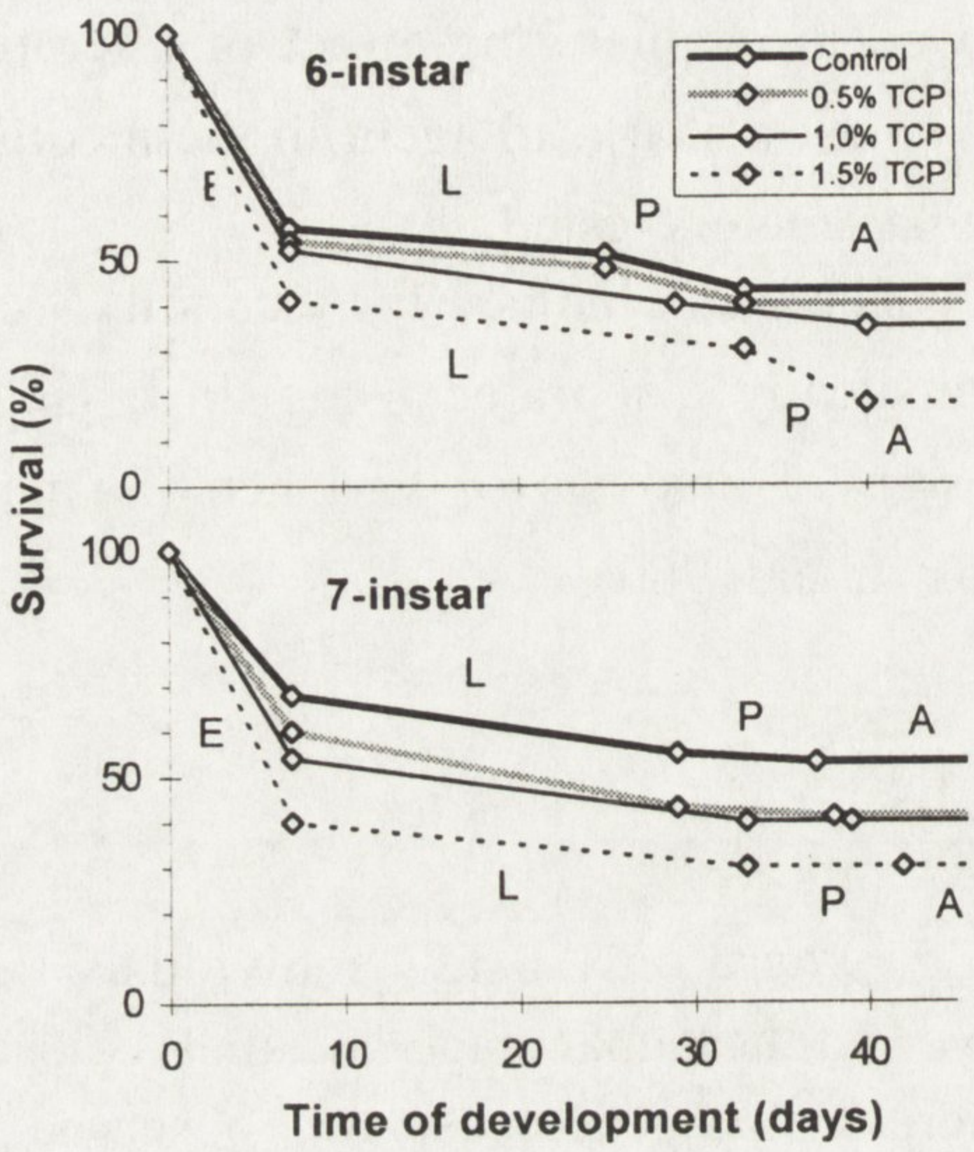

Fig. 2. Survival (%) from egg stage of *T. confusum* 6-instar and 7-instar groups at various concentrations of tricalcium phosphate (TCP). Explantions as in Fig. 1

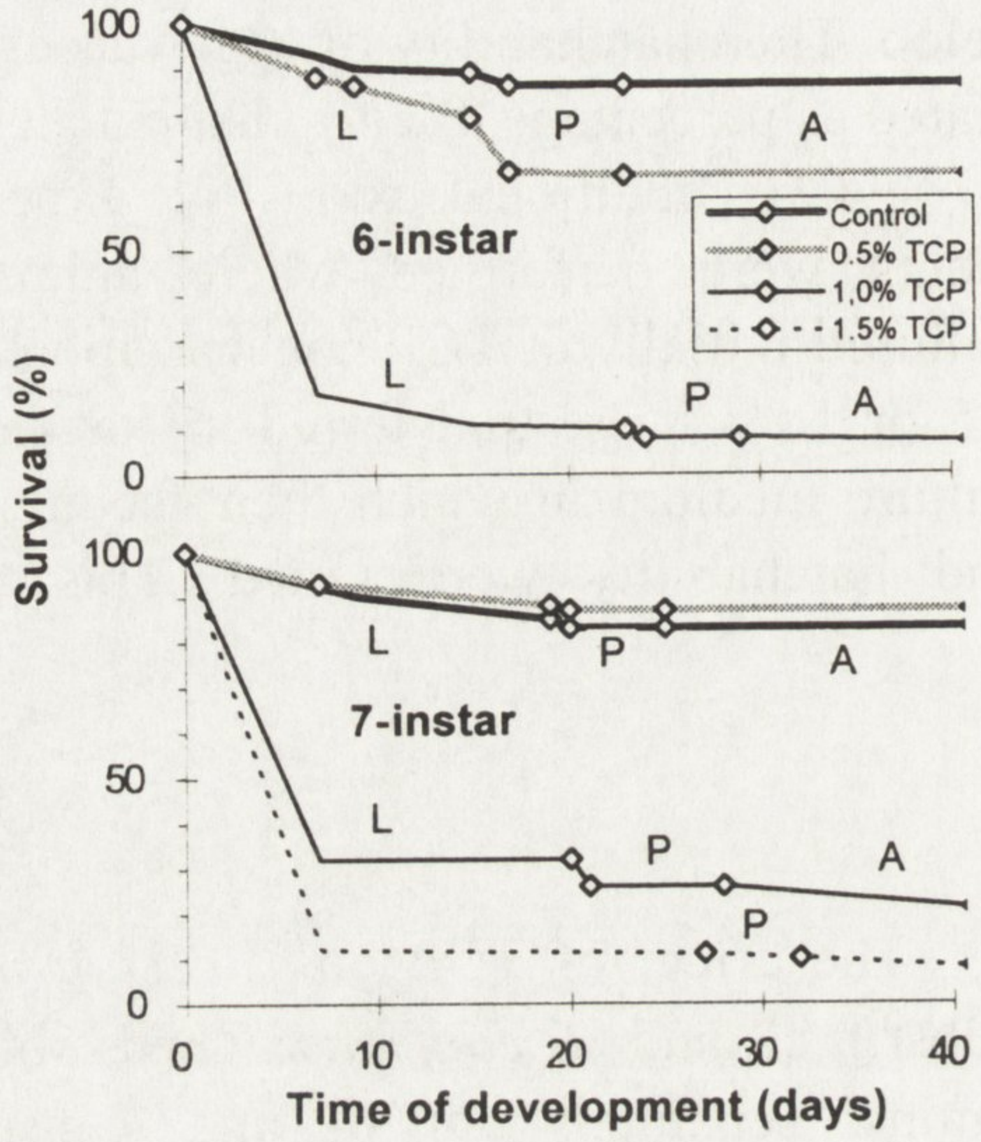

Fig. 3. Survival (%) from newly hatched larvae of *T. confusum* 6-instar and 7-instar groups at various concentrations of tricalcium phosphate (TCP). Explantions as in Fig. 1

is about the same in the two first concentrations, and 25% in 1.5% concentration.

In 7-instar group of *T. confusum* further decrease of larval and pupal stages amounts 15% of controls. The TCP concentrations: 0.5 and 1.0% bring about less than 10% decrease, and concentration 1.5–10% decrease.

The development is prolonged at TCP concentrations in both instar groups by 4–5 days except concentration 0.5% in which it is the same as in controls.

In order to avoid the obscuring effect of differentiated egg hatchability on the results of TCP impact another experiment was performed with newly hatched larvae used at the starting point. In *T. castaneum* (Fig. 3) 6-instar group, the mortality in postlarval stages amounts to about 15% in controls, 35% in TCP concentration of 0.5% and over 90% in 1.0% TCP concentration. A hundred per cent mortality was observed in 1.5% concentration at the beginning of experiment. In 7-instar group the survival of individuals was very high and similar as in control at TCP concentration 0.5%, whereas it was rather poor at 1.0% and very low at 1.5% TCP concentration. The mortality occurred mostly in early larval stages except for 0.5% concentration in both instar groups, where there was either no mortality (in 7-instar) or small one (in 6-instar group).

The similar pattern of delayed effect on developmental time was observed as that in the first experiment. In general, survival of individuals representing both instar groups in *T. confusum* was better than in *T. castaneum,* but the course of mortality shows a similar pattern.

In *T. confusum* (Fig. 4) the TCP concentration of 1.0 and 1.5% caused mortality about 50% in 6-instar group (0.5% brings about slightly higher mortality than in control) and in 7-instar group the mortality is from 35% in 0.5% to 60% in 1.5% TCP concentration. Prolongation of development of individuals representing the two instar groups in *T. confusum* is very clear and its amounts to 6 days to the moment of pupal appearance.

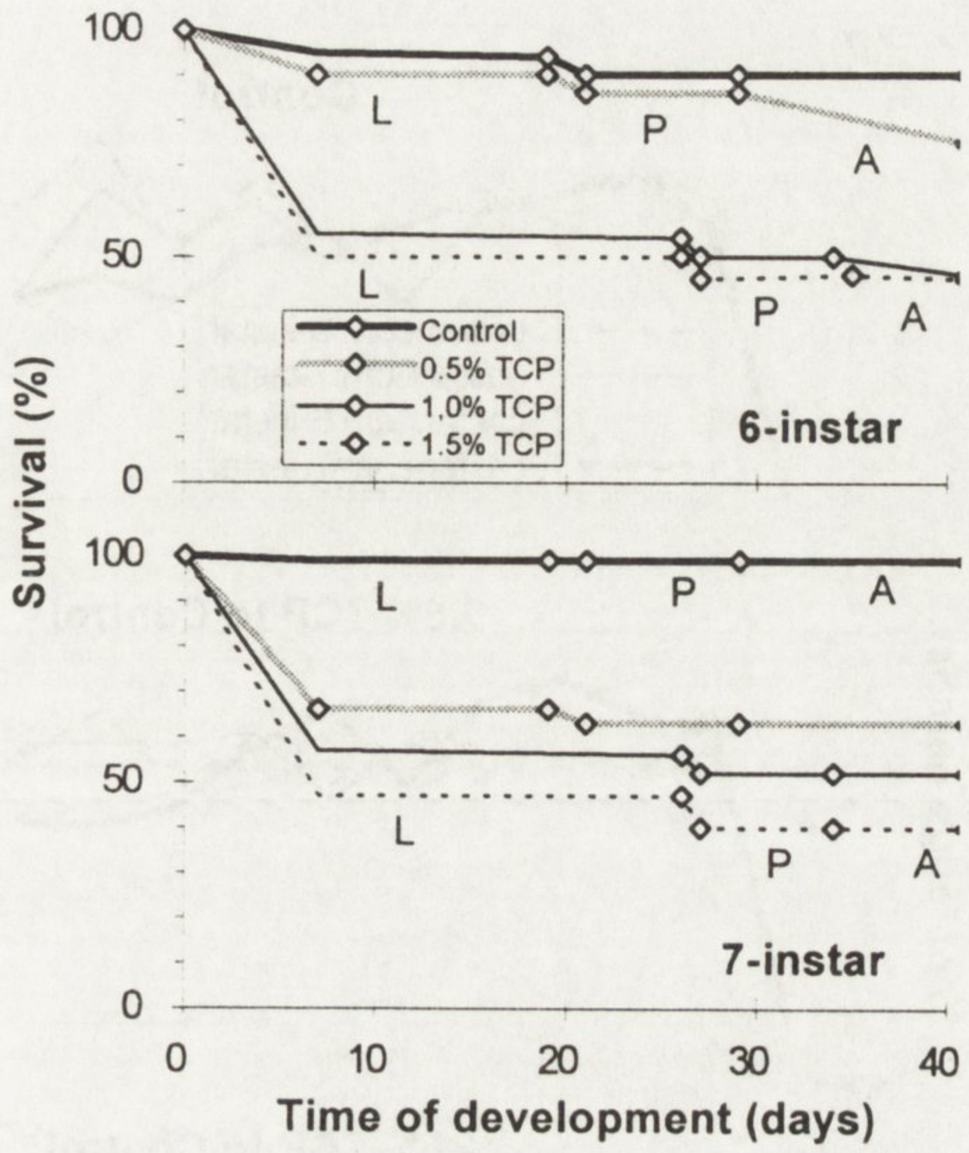

Fig. 4. Survival (%) from newly hatched larvae of *T. confusum* 6-instar and 7-instar groups at various concentrations of tricalcium phosphate (TCP). Explantions as in Fig. 1

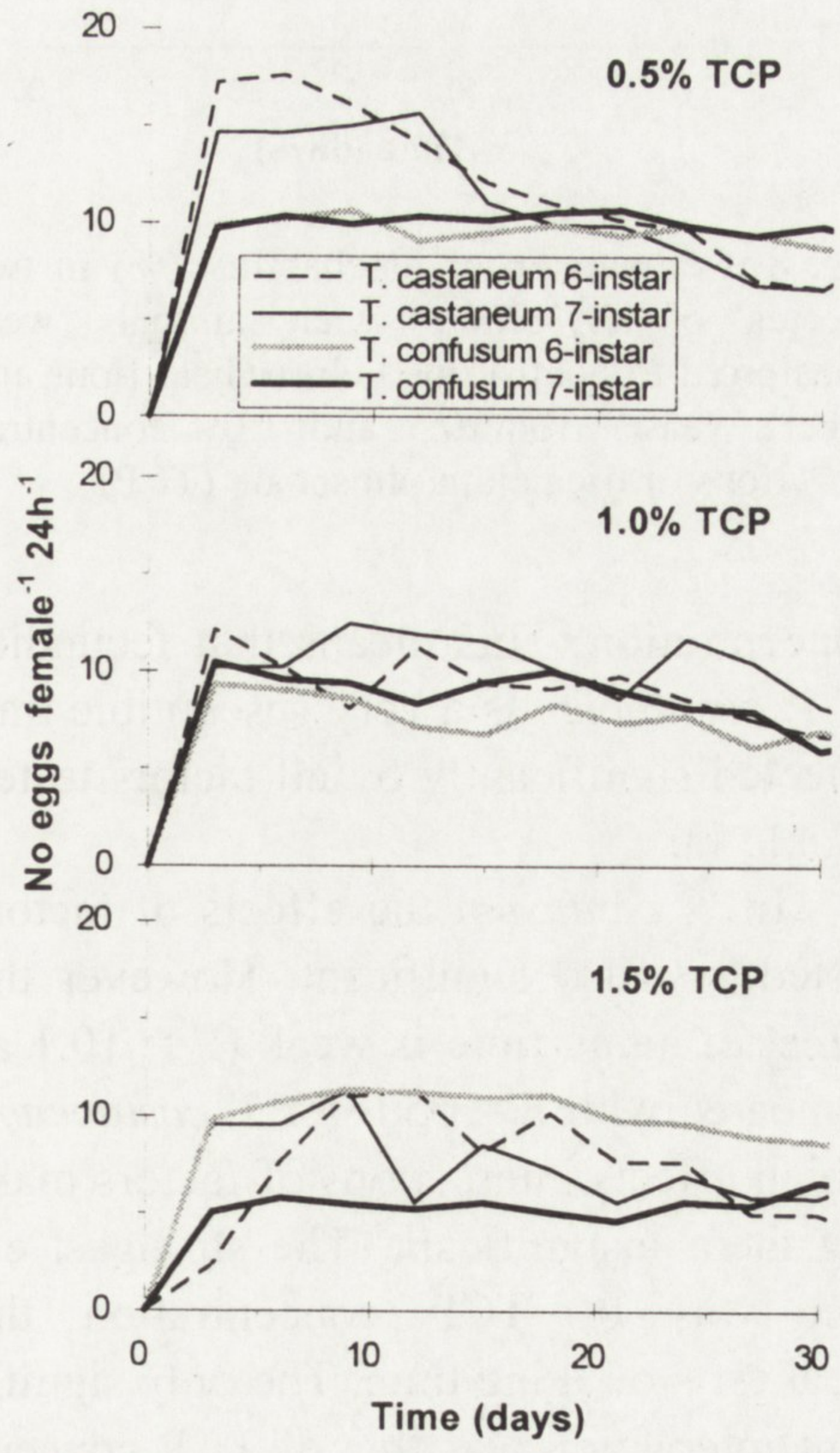

Fig. 5. Comparison of fecundity (number of eggs laid by female per 24 hr) in two species of *Tribolium* in three 0.5, 1.0 and 1.5% concentrations of tricalcium phosphate (TCP) in cultured medium during 30 days of egg laying by beetles

The comparison was made of the effect of TCP on fecundity and hatchability in the two investigated species. In the concentration 1.0 and 1.5% of TCP the rate of fecundity was even, between the discerned groups as well as between both species (Fig. 5). After a rather strong difference occurring at the beginning at concentration 0.5% later on no differences were observed. On the other hand, in the series where fecundity was tested after the transfer of animals from TCP treated medium to pure medium, the species differences were quite evident (Fig. 6) and the value of fecundity came back to the same as in the control medium.

Hatchability being a very variable parameter, does not show any consistent differences within or between species, it

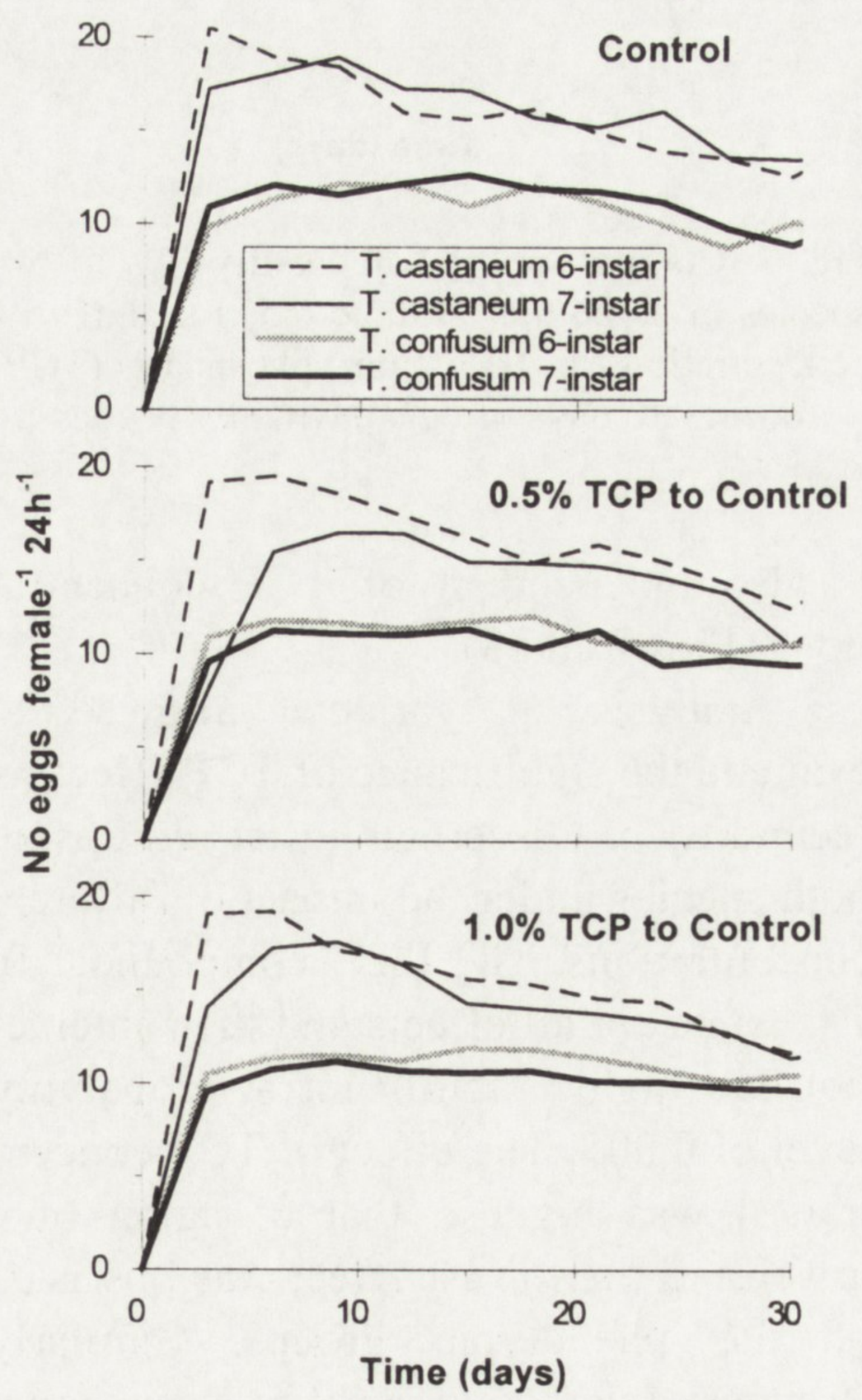

Fig. 6. Comparison of fecundity (number of eggs laid by female per 24 hr) in two species of *Tribolium* when animals were transferred to control medium (wheat flour and baker's yeast) from 0.5 and 1.0 % concentrations of tricalcium phosphate (TCP)

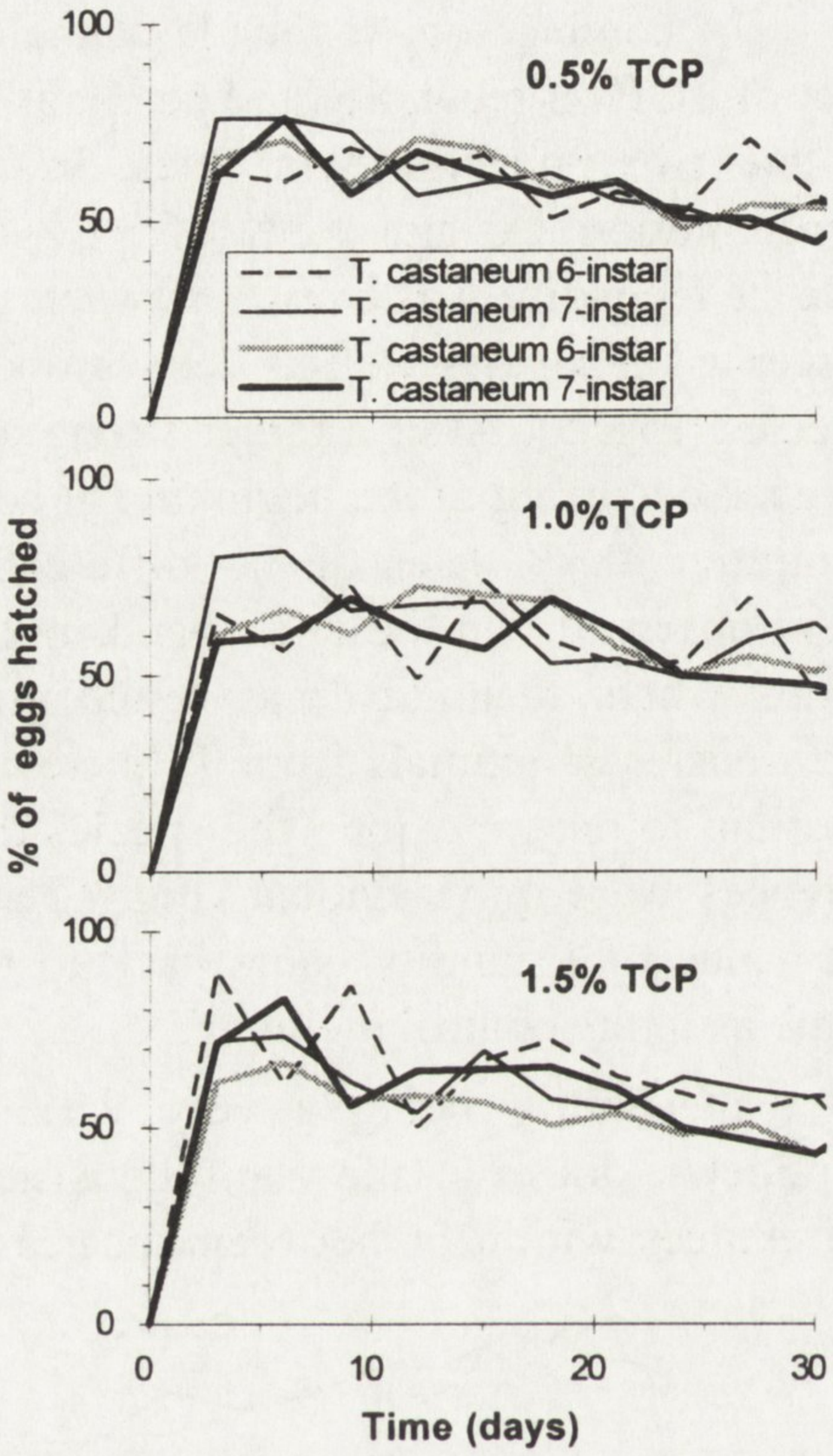

Fig. 7. Comparison of hatchability (%) in two species of *Tribolium* in three 0.5, 1.0 and 1.5% concentrations of tricalcium phosphate (TCP) during 30 days of eggs laying by beetles

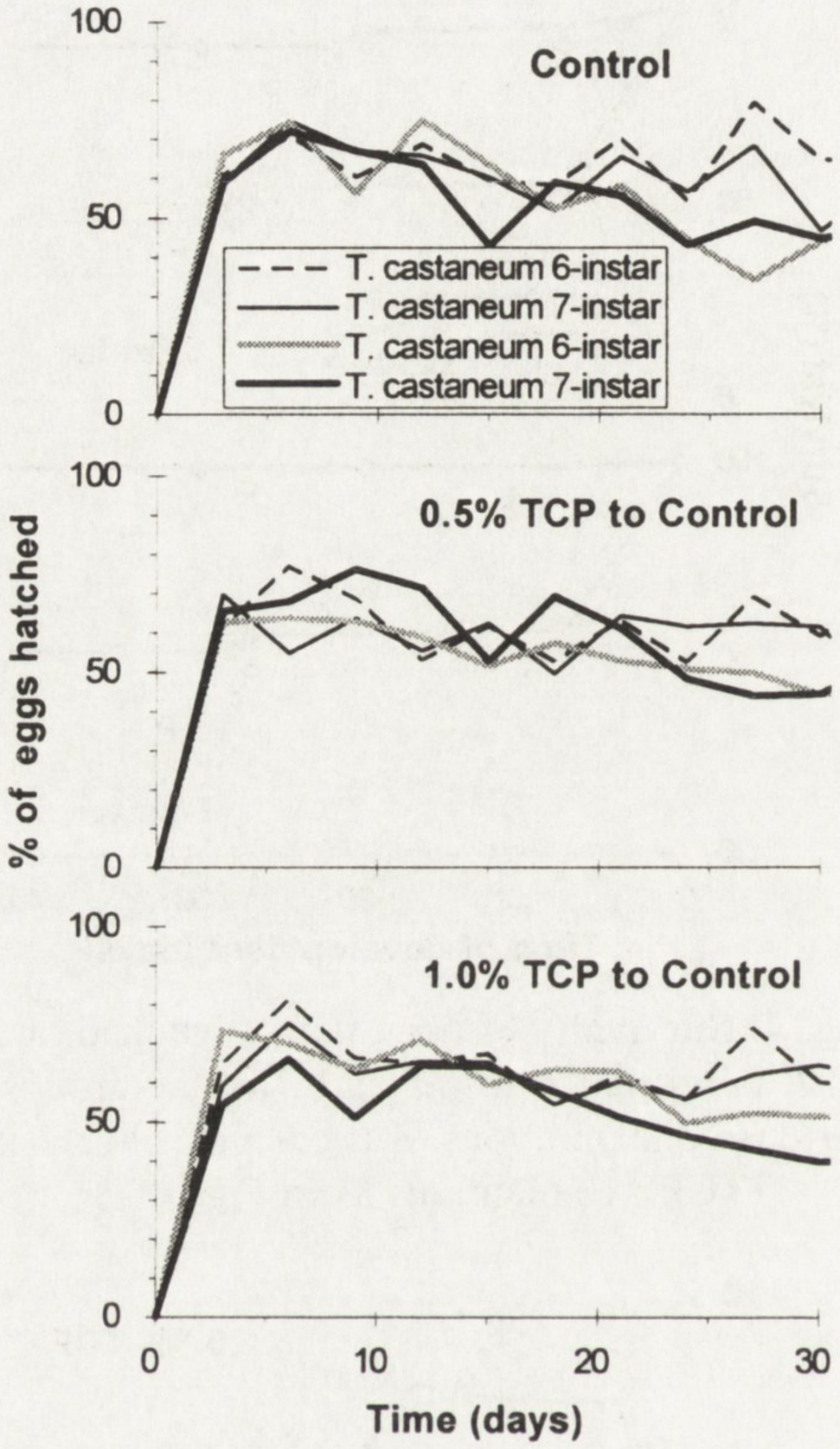

Fig. 8. Comparison of hatchability (%) in two species of *Tribolium* when animals were transferred to control medium (wheat flour and baker's yeast) from 0.5 and 1.0% concentrations of tricalcium phosphate (TCP)

is also independent of TCP concentrations (Figs 7 and 8).

Analysis of variance allowed to evaluate the significance of TCP effect on fecundity of discerned instar groups in both species under the impact of different concentrations of this compound. In *T. castaneum* all effects and their interaction are highly significant at probability level of 0.005. The effect of TCP concentration was highest, that of aging time lower and the lowest effect was observed of the phenotypic groups. Similarly strongest interaction was between aging time and instar group, weaker between TCP concentration and instar group and the lowest between aging time and TCP concentration. This means that fecundity of *T. castaneum* is a very susceptible trait affected significantly by all factors tested (Table 1).

In *T. confusum* the effects of factors tested are also significant. However the effect of aging time is weak (F = 10.1 as compared with F = 55.6 for *T. castaneum*) which affects interactions of factors making them insignificant. The strongest effect was of TCP concentration the weakest – of aging time. The only significant interaction was this of TCP concentration × instar group. This proves that in this species even the fecunditity was independent of time (Table 2).

Table 1. Two-ways ANOVA of fecundity in T. castaneum (Hbst) of 3 parameters: aging time, tricalcium phosphate (TCP) concentration and instar-group effect

Source		Sum of squares	d.f	Mean square	F	P
	aging time effect	4219.3	10	421.9	55.5	0.005
	TCP concentration	7715.2	4	1928.8	253.9	0.005
	6- and 7- instar-group	189.3	1	189.3	24.9	0.005
Interactions	time/concentration	1233.3	40	30.8	4.06	0.005
Interactions	concentration/instar-gr.	337.3	4	84.3	11.1	0.005
Interactions	time/instar-group	1374.2	10	137.4	18.1	0.005
Interactions	time/instar-gr./concentr.	400.2	40	10.0	1.3	0.005
	deviation	7519.2	990	7.6		
	total	22988.0	1099			

Table 2. Two-ways ANOVA of fecundity in *T. confusum* Duval (explanation – see Table 1)

Source		Sum of squares	d.f	Mean square	F	P
	aging time effect	479.1	10	47.9	10.1	0.005
	TCP concentration	2170.0	5	434.0	91.2	0.005
	6- and 7-instar group	156.7	1	156.7	32.6	0.005
Interactions	time/concentration	213.7	50	4.3	0.9	NS
Interactions	concentration/instar-gr.	1195.4	5	239.1	50.4	0.005
Interactions	time/instar-group	32.3	10	3.2	0.7	NS
Interactions	time/instar-gr./concentr.	202.1	50	4.0	0.8	NS
	deviation	5650.6	1188	4.7		
	total	10099.9	1319			

4. DISCUSSION

The experiments presented in previous paper (P r u s 1996) concerned the so-called "natural medium" (i.e. wheat flour in which *Tribolium* species usually live in stores. Such situation is observed only in stores or shops with low temperature (low) and very low humidity where aeration is good. In case when such conditions are not present and, for example, high temperature (about 20–25° C) and high relative (60–70%) humidity is observed, the fungi have very good conditions to develop and enriched environment with vitamins from B group.

It caused the increase of fecundity and hatchability of insects such as *Tribolium*. In the United States of America tricalcium phosphate (TCP) is added to flour to prevent development of strored product pests, *Tribolium* species among them.

All intra – and interpopulation diversity phenomena described recently refer to such medium (Bijok 1986, 1989, Prus 1976, Prus, Prus 1987, Prus et al. 1989). Previous research on the effect of tricalcium phosphate on *Tribolium* strains and their discerned groups was also done using such standard medium (Prus 1989).

The strong effect of TCP on development of *T. castaneum* was observed (Majumber and Bano 1964) in the form of delayed growth and change in body coloration. At 2.0% concentration of TCP exerted strong toxic effect hindering the pupation process and killing adults in the moment of metamorphosis. Bearing all this in mind the concentration of TCP below 2.0% were chosen for the experiments in order to be able to trace any differentiated effect on survival, duration of development, fecundity and hatchability in both groups of the two species.

At much stronger effect of TCP on *T. castaneum* than on *T. confusum*, the examined concentrations of this salt bring about more variable effect in 6-instar group than in7-instar ones. In *T. confusum*, on the other hand, 6-instar group seems to be less vulnerable to harmful effect of TCP, especially at the lowest TCP concentration.

The response to TCP in such conditions differs considerably from that observed earlier for medium without yeast especially as survival and fecundity are concerned. In the medium with yeast admixture, reverse results are observed – *T. confusum* is more depleted by TCP than is *T. castaneum*. In both species in cultured medium deprived of yeast there were no differences between the discerned groups in reaction to TCP. In standard medium (enriched with baker's yeast), on the contrary, the observed differences were significant. The fecundity of both species in pure flour was generally lower than that in the standard medium (Prus 1989, 1990 and 1995).

When no TCP was applied in flour deprived yeast the mortality of larvae during development was lower in both instar groups of *T. castaneum*. In *T. confusum* the mortality in both media was similar, around 50% at the end of development.

The observed fact of diminishing survival with simultaneous increase in fecundity at different culture media for *T. confusum* or reverse situation observed in *T. castaneum* (Prus 1995) corroborates the well known phenomenon that increasing one component of fitness, e.g. fecundity, often leads to a decrease in another e.g. survival". Such compensation is the main mechanism of realisation of life history strategy.

All eperiments compared here were carried out on the same strains: *T. castaneum* cI and *T. confusum* bIV.

Out of proposed by Reznick (1985) four possible methods to describe trade off (phenotypic correlation, experimental manipulation, genetic correlation and artificial selection experiments) the experimental manipulation (by changing the food conditions and introducing an impact of toxicant) was applied here. It yielded results which corroborated the presumption on survival – fecundity relationship.

In addition, the present and previous results proved that hatchability is not an adequate feature to be included in fitness studies on account of its extraordinary

variation within treatments. Higher hatchability in pure flour as compared to that in standard medium is difficult to explain. The possible reason that is yeast enhances the fecundity which in turn increases the production of less viable eggs.

Another way used for control of stored product pests is based on treatment of animals with toxins extracted from plant. For example, toxicity of vapour oil of *Acorus calamus* L. is commonly used as inhibitor for immature and mature stages of some stored product pests of Coleoptera. However, this toxic substance had no effect on immature and mature stages of *T. confusum* and several other species (R i s h a et al. 1990).

In countries where another plant species *Annona squamosa* Linn. is widely distributed (tropical America, India and southeast Asia) its dried seeds, leaves or fruits are commonly used instead of insecticides. Especially seeds and roots of *Annona squamosa* Linn. contain an insecticidal substance which, when concentrated with ether becomes an active agent against several insect species. Addition of *Annona squamosa* seed oil extracted with petroleum ether to culture medium caused significant reduction of adult emergence rate from the larvae treated with this substance. Highly significant reduction in weight of pupae and adults of *T. confusum* was also observed. This substance also caused prolongation of the pupal stage in this species. (P a r v e e n and S e l m a n 1995a, b). But in these two examples decribed above there is nothing know about constant or temporary impact of these substances on studied parameters especially on fecundity and hatchability.

In both species and both discerned instar groups the transfer of animals from medium with TCP to control medium caused recovery of fecundity, practically to the same level as in controls. The results allow to conclude that the temporary impact of TCP on fecundity is a recuperable phenomenon. Once the impact ceases to affect (transfer of beetle to control medium after exposure to TCP) the pattern of fecundity in both species regains its original level. This may suggest that if TCP used as protecting factor for wheat flour, it should be constantly present in it, otherwise the suppression of granary pests due to temporary treatment will be not effective.

Treatment with TCP will be also not effective if conditions in stores and shops are unadequate differ from these ones proposed by E v a n s (1987) i.e. at low temperature and very low humidity.

5. SUMMARY

Tricalcium phosphate diminishes survival and prolongs developmental time in both species of *Tribolium*.The same differences were found between 6- and 7-instar groups.

The effect of TCP on survival is stronger in *T. castaneum* than that in *T. confusum* (Figs 1 and 2). The same results were observed in experiments which started from newly hatched larvae (Figs 3 and 4).

Tricalcium phosphate diminishes also fecundity proportionally to its concentration in the medium. The diminishing is stronger in *T. castaneum* than in *T. confusum* (Fig. 5).

No regular effect of TCP was observed on hatchability of eggs in all treatments (Fig. 7).

Transfer of animals from TCP treated medium to control conditions brings about fast recuperation of fecundity (Fig. 6) and no changes were observed in hatchability (Fig. 8).

This proves that TCP is an efficient factor to depress *Tribolium* development in wheat flour when it is present in the flour constantly, and when the temperature and relative humidity are kept in the range below 30–30° C and below 65%, respectively.

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