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INTRA- AND INTERPOPULATION RESPONSE TO TRICALCIUM PHOSPHATE IN *TRIBOLIUM* STRAINS

ABSTRACT: The experiments were carried out on two species: *Tribolium castaneum* cI Hbst. and *T. confusum* bIV Duval. Within these two species two groups of 6- and 7-instar group 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 the same.

KEY WORDS: Tricalcium phosphate, survival, fecundity, hatchability,*Tribolium*

1. INTRODUCTION

The post-harvest crops and foodstuff losses caused by stored product pests in general, and especially by insects reach on average 20%, ranging from 9% in the USA to 50% in some developing countries. These losses are caused not only due to consumption of stored food by pests but also by contaminating these food with faeces and molts, by increasing their humidity, overheating etc. Due to the disadvantages of using insecticides and their harmful effect on human health, it has been necessary to search for other effective substances of pest control.

Food for stored product pests is one of an important ecological factor which limits the population numbers. Insects need not only the presence of particular components in the diet for normal growth and development, but also suitable proportions of these components. The absence of one or more components in the insect diet causes a disturbance in populations such as delay of development,

a change in fecundity and hatchability. Pratt et al. (1972) reported that the use of mineral salts offers a new promising method for insect control. Later on, it was found that the strongest inhibitory effect for stored product pests was that of ammonium nitrate and tricalcium phosphate (Boczek 1984). The tricalcium phosphate (TCP) has proven to be a good source of calcium and phosphate for man and domestic animals and at the same time a 1–3% concentration of this salt is enough to protect the stored flour and grain from the insect infestation.

In this paper the impact of this salt on survival, developmental time, fecundity and hatchability in two species and discerned within both species 6- and 7-instar groups, were examined.

2. MATERIAL AND METHODS

Experiments were carried out on two strains of *Tribolium*: *T. castaneum* cI Hbst and *T. confusum* bIV Duval, both originating from the University of Chicago. In each strain 6- and 7- instar groups were discerned and cultured at 29° C and relative humidity 75% in a dark incubator, cultured medium was wheat flour. 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 pure medium (control) and medium with various concentration of TCP a hundred newly laid eggs were placed. After hatching the eggs, 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 experiment 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 a single pairs in 8 g of either pure 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 develop. Then hatchability of eggs was estimated as percentage of eggs that hatched.

Two additional series of experiments were performed where animals cultured initially in TCP concentration of 0.5 or 1.0% were transferred to pure 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). The computer program specially outlined for these data by Dr P. Bijok in the Institute of Ecology was used.

3. RESULTS

The effect of TCP on *T. castaneum* cI and *T. confusum* bIV strains separately for 6- and 7-instar groups can be presented in the form of survival curves for each concentration of this salt (Figs. 1 and 2). In *T. castaneum* 6- and 7-instar groups show the same response to various concentrations of TCP. With increasing concentration of this salt the decrease in survival was observed. Considering the duration of development it is prolonged by about 2 days at the medium at concentrations 1.0 and 1.5% (Fig. 1). Similarly in *T. confusum* 6- and 7-instar group reaction to various

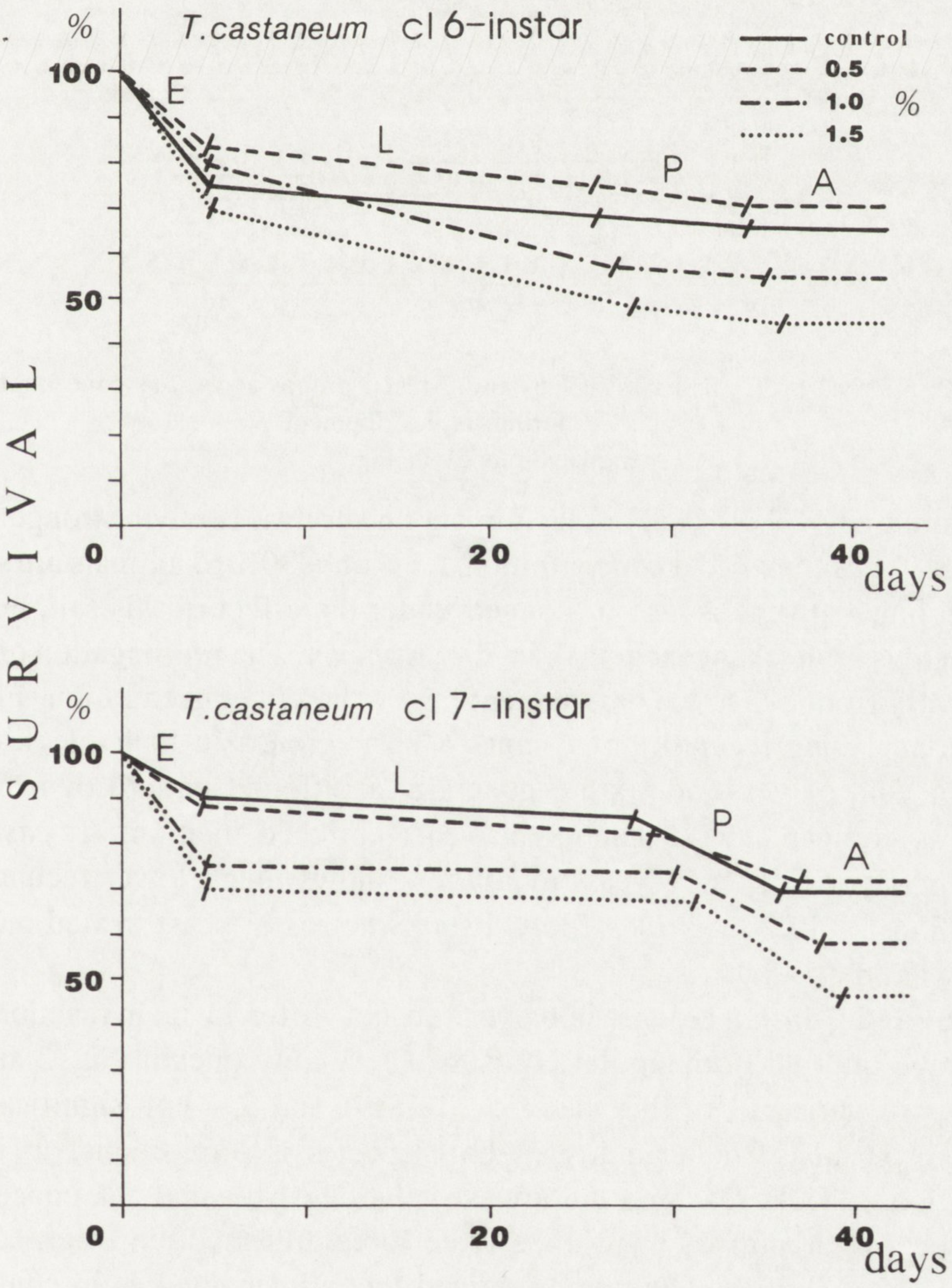

Fig. 1. Survival of *T. castaneum* cI 6- and 7-instar groups at various concentrations of TCP during its development. E – eggs, L – larvae, P – pupae, A – adults

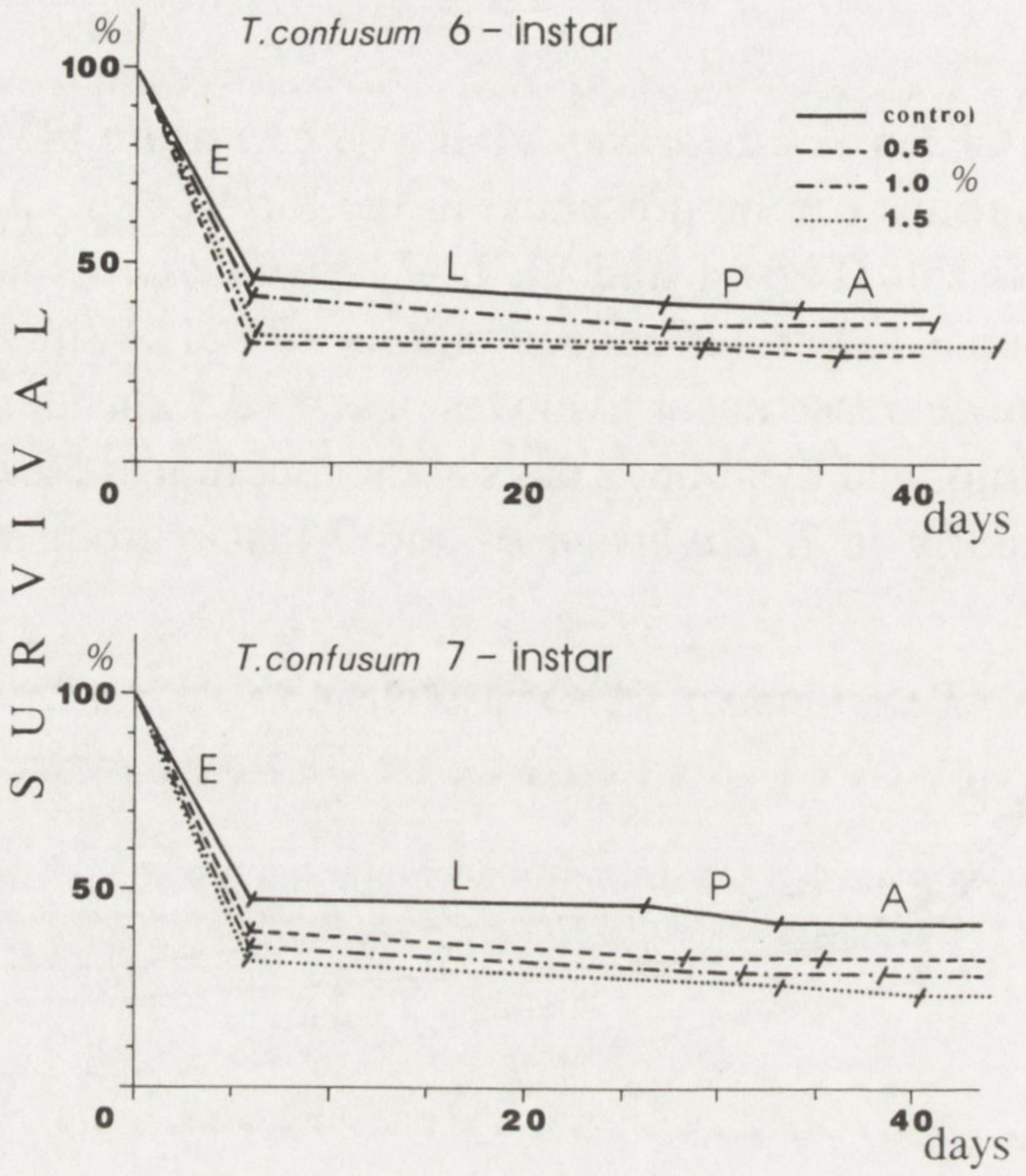

Fig. 2. Survival of *T. confusum* bIV 6- and 7-instar groups at various concentrations of TCP during its development
Explanations as in Fig. 1

concentrations of TCP is alike, but the impact on survival is much stronger than that observed in *T. castaneum*. At concentration 1.5% only 30% of animals are surviving. The developmental time is also prolonged under the effect of this salt, but span of development is more changeable than in *T. castaneum*. The prolongation of development can vary from two to ten days depending on the salt concentration (Fig.. 2).

When analysing fecundity of 6- and 7-instar groups in both species cultured in medium void of yeast admixture generally a different pattern of reaction was observed as compared with that in the yeast enriched medium. *T. castaneum* – a better egg-layer in flour + yeast medium – shows much lower fecundity in all concentrations of TCP than does *T. confusum* whereas in yeast treated medium the opposite was true.

The 6- and 7-instar groups, however, do not differ in their reaction to TCP, which can be inferred from similar courses of fecundity lines in Fig. 3 and proved by analysis of variance for this material (Table 1 and 2 – non significant effects of ecotype). Decrease in fecundity of both species is proportional to increasing concentration of TCP, reaching the lowest values in 1.0 and 1.5% concentrations.

In series when animals were transferred to control medium from 0.5 or 1.0% concentration of TCP the fecundity regained the similar level as in controls, irrespective to the concentration at which the development of animals took place previously (Fig. 4).

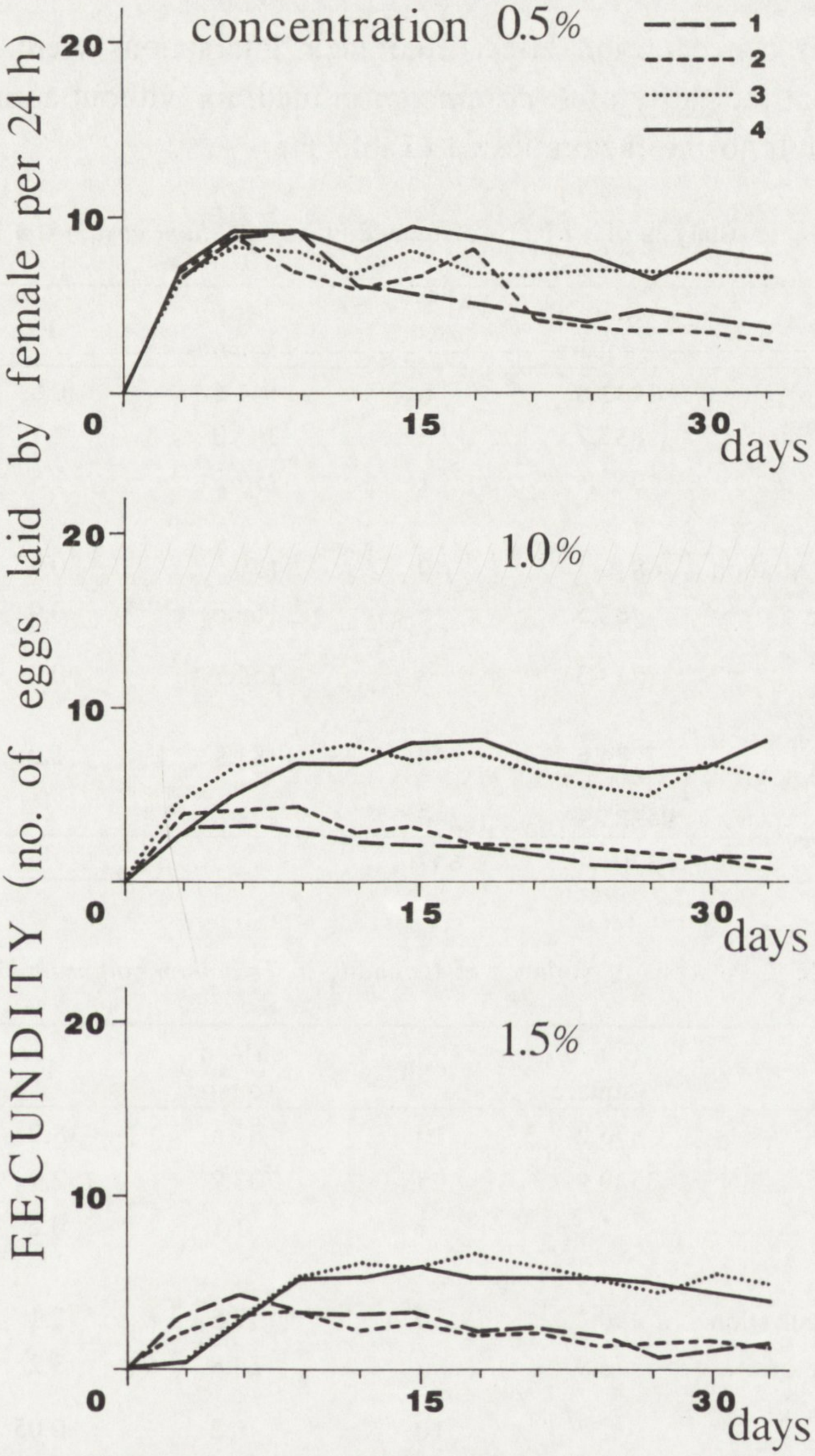

Fig. 3. Comparison of fecundity (number of eggs laid by female per 24 h) in two species of *Tribolium*

1 – *T. castaneum* cI 6-instar group; 2 – *T. castaneum* cI 7-instar group; 3 – *T. confusum* bIV 6-instar grup; 4 – *T. confusum* bIV 7-instar group

Hatchability, being most variable population trait in *Tribolium* does not show any consistent changes between instar groups or species. It is also independent of TCP concentration (Fig. 5 and 6). Generally, hatchability of eggs laid in flour without yeast was much better (around 70%) than that in yeast treated medium (ca 50%). It was still high at TCP concentrations.

Analysis of variance allowed to evaluate the significance of TCP effect on fecundity in different groups discerned in the two species. In *T. castaneum* all

effects (except concentration effect) and their interactions were nonsignificant. This means that fecundity of *T. castaneum* in medium without admixture of yeast is not susceptible to the factors tested (Table 1).

Table 1. Analysis of variance of fecundity in *Tribolium castaneum* Hbst.

Source	Sum of square	df	Mean squares	F	P
Time effect	1132.8	8	141.6	0.8	NS
Concentration	1835.2	5	367.0	2.1	< 0.05
Instar group	257.3	1	257.3	1.5	NS
Interactions:					
time x concentration	6819.4	40	170.5	0.9	NS
time x instar group	782.5	5	156.6	0.9	NS
concentration x instar group	1213.3	8	151.7	0.9	NS
time x concentration x instar group	7274.6	40	181.9	1.0	NS
deviations	94385.9	540	174.8		
Total	113701.1	647			

Table 2. Analysis of variance of fecundity in *Tribolium confusum* Duval.

Source	Sum of square	df	Mean squares	F	P
Time effect	876.2	10	87.6	18.9	< 0.005
Concentration	3539.9	5	707.9	152.9	< 0.005
Instar group	1.1	1	1.1	0.2	NS
Interactions:					
time x concentration	490.2	50	9.8	2.1	< 0.005
time x instar group	73.9	5	14.8	3.2	< 0.01
concentration x instar group	2.5	10	0.2	0.05	NS
time x concentration x instar group	169.1	50	3.4	0.7	NS
deviations	4889.6	1056	4.6		
Total	10042.6	1187			

In *T. confusum* the effect of time and concentration of TCP were significant, whereas ecotype effect was insignificant. In this analysis only interaction time x concentration was significant (Table 2). Thus, *T. confusum* when cultured in flour without admixture of yeast reveals much higher susceptibility to TCP than *T. castaneum*.

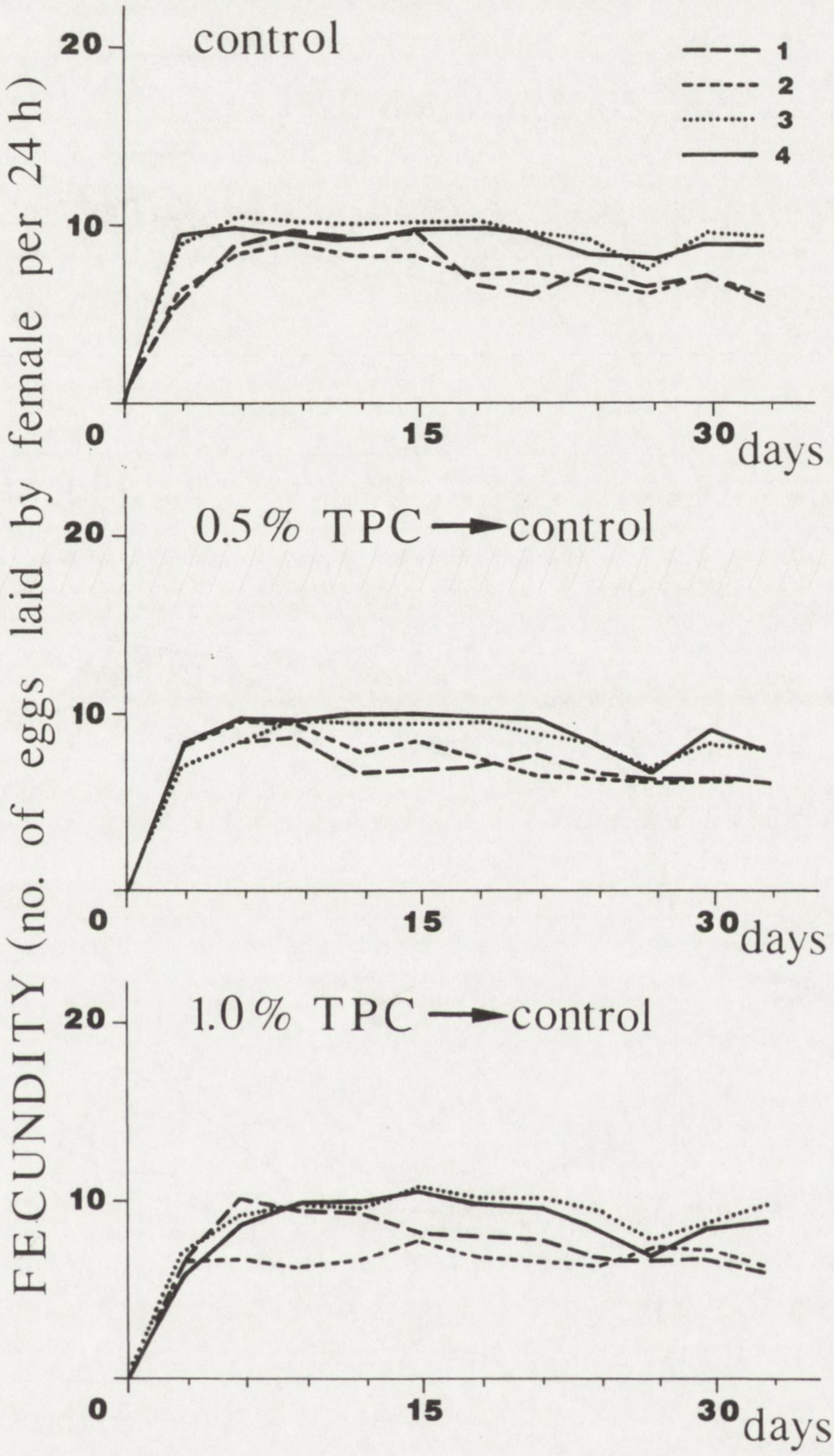

Fig. 4. Comparison of fecundity (number of eggs laid by female per 24 h) in two species of *Tribolium* when animals were transferred to control medium from various concentrations of TCP
Explanations as in Fig. 3

4. DISCUSSION

Hitherto all the investigations performed with *Tribolium* strains such as populational, physiological, genetical and bioenergetical ones were carried out in so-called standard medium which consisted of 95% of wheat flour and 5% of baker's yeast by weight. Thus all intra- and interpopulation diversity described recently refers to such medium (Bijok 1986, 1989, Prus 1976, Prus and

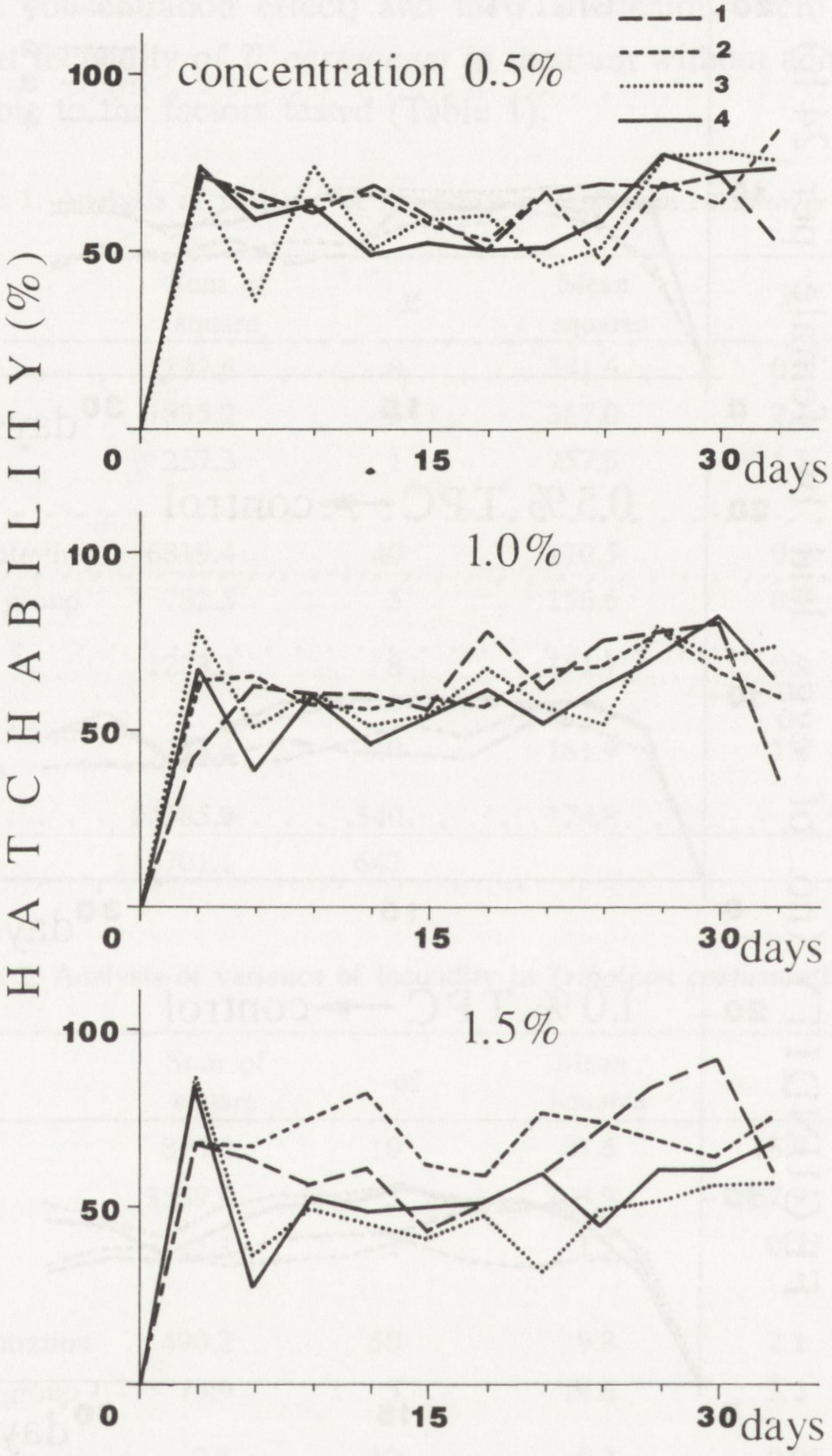

Fig. 5. Comparison of hatchability (%) in two species of *Tribolium*
Explanations as in Fig. 3

Prus 1987, Prus et al. 1989). Previous research on the effect of TCP on *Tribolium* strains and their discerned groups was also done using such standard medium (Prus 1989, Prus, in prep.). The experiments presented in the present paper concerned the so-called "natural medium" (wheat flour) in which these stored product pests usually live in stores.

The response to TCP in such conditions differs considerably from that observed earlier for standard medium especially as survival and fecundity are concerned. In the medium without yeast admixture, *T. confusum* is more depleted

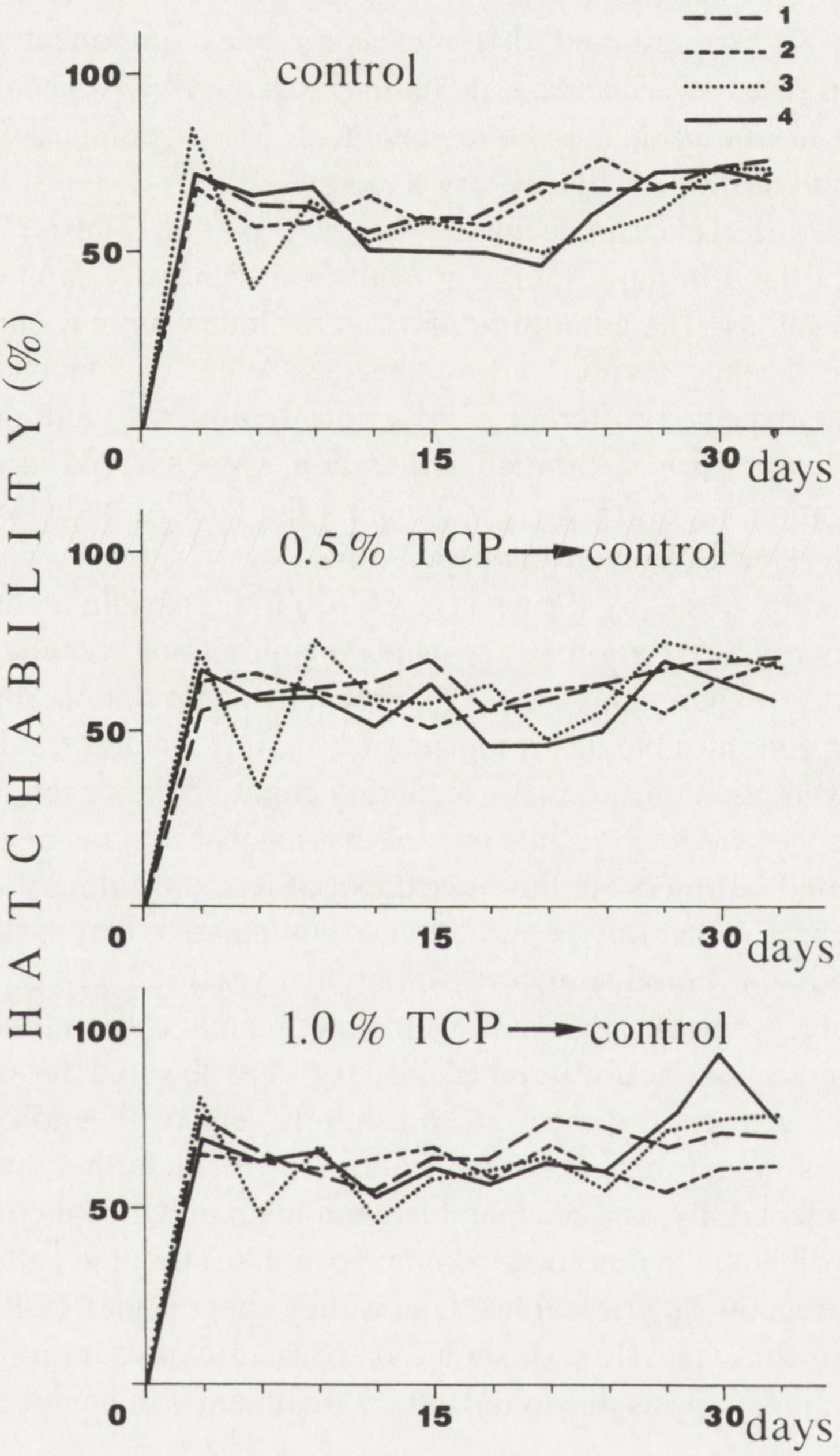

Fig. 6. Comparison of hatchability (%) in two species of *Tribolium* when animals were transferred to control medium from various concentrations of TCP
Explanations as in Fig. 3

by TCP than that of *T. castaneum* whereas reverse was observed in the standard medium. In the two species there were no differences observed between the discerned groups. In standard medium, on the contrary, the observed differences were significant (Prus 1989). The fecundity of both species in pure flour was generally lower than that in the standard medium.

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* corroborates the thesis reported by Møller et al. (1989). These authors stated: "It is presumed that increasing one component of fitness, e.g. fecundity, often leads to a decrease in another e.g. survival". The obtained results are very much in line with the above statement. Such compensation is the main mechanism of realisation of life history strategy.

Obliterated differences in fecundity between 6- and 7-instar groups in both species when cultured in pure flour can result from generally suppressed fecundity under such conditions. The admixture of yeast to flour was introduced many years ago to enhance the egg laying by flour beetles. When the fecundity is rised by this stimulus the expected differences in various treatments yield positive answers when tested for significance. Similar obliteration was observed in treatments with different concentrations of TCP. This explains the lack of statistically significant differences described earlier in this paper.

It seems that 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) yielded results which corroborated the presumption on survival – fecundity counterbalance relationship.

In addition the presented 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. Introducing a reasoning that yeast induced fecundity resulted in production of less vitable eggs claims for experimental elaboration.

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 pure flour after exposure to TCP) the pattern of fecundity in both species regains its original level. This may suggest that TCP if to be 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.

5. SUMMARY

Tricalcium phosphate diminishes survival and prolongs developmental time in both species of *Tribolium* whereas no such differences were found between 6- and 7-instar groups.

The effect of TCP on survival is stronger in *T. confusum* than that in *T. castaneum*. (Fig. 1, 2).

Tricalcium phosphate diminishes also fecundity proportionally to its concentration in the medium. The diminishing is stronger in *T. castaneum* than in *T. confusum* (Fig. 3, Table 1 and 2).

No regular effect of TCP was observed on hatchability of eggs in all treatments (Fig.5 and 6).

Transfer of animals from TCP treated medium to control conditions brings about fast recuperation of fecundity (Fig. 4).

This proves that TCP is an efficient factor to depress *Tribolium* populations in wheat flour when it is present in the flour constantly.

6. POLISH SUMMARY

Dodanie do pożywki trójwapniowego fosforanu (TCP) w różnych stężeniach powoduje obniżenie przeżywalności chrząszczy i wydłuża ich czas rozwoju u obu gatunków *Tribolium*. Nie stwierdzono różnic w przeżywalności i w wydłużeniu czasu rozwoju pomiędzy grupami 6- i 7-stadialnymi. Wpływ TCP na przeżywalność jest silniejszy u *Tribolium confusum* niż u *Tribolium castaneum* (rys. 1, 2).

Trójwapniowy fosforan powoduje również spadek płodności. Obniżenie płodności jest proporcjonalne do stężenia TCP. Stwierdzono silniejszy wpływ trójwapniowego fosforanu na płodność u *T. castaneum* niż u *T. confusum* (rys. 3, tabela 1 i 2).

Nie stwierdzono istotnego i ukierunkowanego wpływu TCP na zdolność wylęgową jaj u obu gatunków i obu grup fenotypowych (rys. 5 i 6).

Przełożenie zwierząt ze środowiska zawierającego TCP do czystej mąki (kontrola) powoduje poprawę płodności *Tribolium* prawie do wartości stwierdzanych w kontroli (rys. 4).

Otrzymane wyniki wskazują na to, że TCP jest dobrym inhibitorem obniżającym w znacznym stopniu płodność szkodników magazynowych ale tylko w przypadku jego stałej obecności w środowisku.

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