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Effects of herbicides Paraquat and Atrazine upon Collembola, Folsomia candida (Willem) and Tullbergia granulata Mills

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EFFECTS OF HERBICIDES PARAQUAT AND ATRAZINE UPON COLLEMBOLA, *FOLSOMIA CANDIDA* (WILLEM) AND *TULLBERGIA GRANULATA* MILLS

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ABSTRACT

EFFECTS OF HERBICIDES PARAQUAT AND ATRAZINE UPON COLLEMBOLA, FOLSOMIA CANDIDA (WILLEM) AND TULLBERGIA GRANULATA MILLS

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Folsomia candida (Willem) and Tullbergia granulata Mills were fed on powdered brewer's yeast treated with herbicide. Three concentrations of Paraquat and Atrazine (600 ppm, 1000 ppm, and 5000 ppm) were tested to evaluate the effects on Collembola. Cultures were reared at 60° F and observed daily for 22 weeks.

Collembola fed on herbicides significantly decreased their egg production and delayed the hatching time of their eggs. Feeding on Atrazine also prolonged instar duration. Only 5000 ppm of Paraquat lengthened the instar duration of F. candida, and was not significant at lower doses. However, the instar duration of T. granulata was significantly extended by feeding on Paraquat. Results suggest that feeding on herbicides affect the reproductive system. Food treated with 5000 ppm of Atrazine may be less palatable to Collembola; in addition high mortalities were observed at this concentration

The possibility of direct lethal effects were also tested. The Collembola were reared on soil sprayed with herbicide at field application dosage. The concentrations were 600 ppm for Paraquat, and 5000 ppm for Atrazine. Apparently, Paraquat does not have directly

lethal effects on Collembola. However, Atrazine shows notable effects.

The results of these experiments indicate that Atrazine produces more severe effects as compared to Paraquat.

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INTRODUCTION

Herbicides are destructive chemicals commonly applied to control weeds in agricultural situations. Application of these chemicals may contaminate plant remains, and as a result, may be toxic to soil animals. Furthermore, elimination of the plants could reduce the source of food for much of the soil fauna. Therefore, application of herbicides may influence the soil community.

In 1952, Baudissin made an investigation on Collembola and Acari in a field treated with 2,4-D. He found no effect of the herbicide on the populations of Collembola and Acari. Since that time, the influence of herbicides on soil animals has attracted much interest among scientists. Effects caused by herbicides on soil fauna still are not completely understood, even though many studies have been conducted.

Among the arthropods, the insect order Collembola has the greatest number of soil species besides Acari. They play an important role in the breakdown of dead plants, and are among the most important producers of humus (Schaller, 1968). Field investigations have shown that some herbicides affect the populations of Collembola (Edwards & Thompson, 1973). Some herbicides are indeed directly toxic to these animals. However, in the field, the influence is more indirect than direct (Edwards, 1969; 1970; Edwards & Thompson, 1973).

Laboratory studies are very helpful in understanding the direct toxic effects. Study on the direct exposure of 2,4,5-T to *Onychiurus quadriocellatus* Gisin, has provided valuable information on the poisoning process of herbicides on Collembola. The Collembola are paralyzed before dying (Eijsackers, 1975).

The present study was conducted to ascertain some biological aspects of Collembola reared under direct feeding on herbicides, and if field applications of herbicides have lethal effects on the soil animals. This type of study is, at present, not commonly undertaken. Therefore, this study is viewed as a basis for future investigations.

REVIEW OF LITERATURE

Effects of herbicides on soil animals have been investigated primarily in field situations, at the "group" level. Only a few laboratory experiments were conducted at the species level.

In fields, effects are highly variable and depend on a large number of factors, i.e.: the diversity of soil fauna, soil condition, weather and time of application, method of applications, and persistence of their residues on soil (Edwards & Thompson, 1973). This review is mainly concerned with the influence of herbicides on Collembola. Under some circumstances, however, discussion of arthropods and other soil animals are given in this review.

Effect on Density

Studies by Baudissin (1952), Rapoport & Canglioli (1963), Fox (1964) and Prasse (1975) concluded that Collembola populations were not affected by application of 2,4-D. Application of MCPA (4-chloro-2-methylphenoxyacetic acid) did not change the populations of Collembola (Rapoport & Canglioli, 1963; Edwards & Arnold, 1964). This was confirmed by Davis (1965) with a study in a field that had been treated annually for a ten out of thirteen year period with MCPA. He concluded that there was no immediate or long term effect on Collembola populations. The same results were also concluded when Edwards and Arnold (1964) tested Linuron and Triallate. Later, Edwards et al.

(1974; 1975) found that the herbicide benzol-prop-aethyl slightly decreased the number of Collembola; however, there was no effect on the total soil fauna. Recent studies with Banvel-D applied in paddy fields and grassland concluded that there was no direct effect on Collembola, but it decreased the total number of soil arthropods (Bhattacharya & Joy, 1977).

There are discrepancies as to the influence of Paraquat and Dalapon. Curry (1970) reported that application of Paraquat in grassland decreased populations of Collembola and Acari, while Edwards et al. (1972) found more arthropods and fewer predatory Acari in the treated plots. Earlier, Edwards (1970) observed that Paraquat had no apparent effect on small invertebrates. Although Fox (1964) found significant increases of Collembola in grassland treated with Dalapon, Curry (1970) noted the opposite effect.

Ostensibly, TCA (trichloroacetic acid) allowed an increase of Collembola populations (Fox, 1964). On the other hand, DNOC (2-methyl-4,6-dinitrophenol) and Bladex decrease the number of Collembola and Acari (Johnson et al., 1955; Edwards et al., 1961; 1971; 1974). Decrease in number of isotomid Collembola was found after application of Shell WL 19805 (Edwards, 1970).

Fox (1964) observed soil fauna populations in grassland for three and four months after application of Atrazine, while Popovici et al. (1977) studied corn field for four months after application. Both studies agreed that Atrazine decrease collembolan populations. A series of observations at Rothamsted Experimental Station showed numbers of Collembola and other soil animals diminished greatly by application of Simazine. The effects were still obvious after six years (Edwards et al., 1964; 1965; 1970). In arable soil, significant lessening of Collembola after application of Simazine was noted by Prasse (1975).

The possibility of using herbicides for controlling collembolan pests as well as weeds was attempted by Marshall and Ilnytzky (1976). They applied herbicide mineral oil, Shell AWK, in Sitka spruce and western hemlock nurseries, for controlling weeds and Bourletiella hortensis (Fitch), a collembolan pest in some agricultural crops. Their study found that this herbicide only temporarily suppressed the pest and concluded that as a pest control, it is not effective.

Obviously, application of herbicides affects populations of Collembola. Although other herbicides prolong the effect, this is generally considered as temporary. Persistence of residues in soil is the most important factor in lengthening influence on animals (Edwards, 1973). Organisms that live in soil with more organic matter will suffer potentially drastic effects, because organic matter absorbs much of the herbicides. In this case, residual effect will last for a longer period (Bhattacharya & Joy, 1977). Discrepancies between results of observers, therefore, is not surprising since soil type and experimental conditions may vary.

Effect on Diversity

Observations on multiformity of Collembola showed that 2,4-D and MCPA did not affect diversity (Baudissin, 1952; Rapoport & Canglioli, 1963; Davis, 1965). Evidently, reduction of population numbers does not always change diversity. Application of Paraquat and Dalapon decreased numbers of Collembola; however, it did not affect diversity (Curry, 1970).

Studies on Collembola in arable soil treated with 2,4-D and Simazine yield distinct results. Prasse (1975) found that after application of 2,4-D, there was more positive shifting dominance than negative shifting. This means that more species increased in number than decreased. Among them were Tullbergia krausbaueri (Börner), Folsomia sp., and Isotoma notabilis Schäffer. After application of Simazine, on the other hand, there was more negative shifting dominance. Lessening of total Collembola was followed by emergence of some species; they were Isotomodes productus Linnaniemi, Pseudonurophorus isotoma Börner, Lepidocyrtus cyaneus Tullberg, and Onychiurus armatus (Tullb.) Gisin. The most drastic reduction occurred with the species Pseudosinella alba (Packard), while Folsomia sp. decreased in all layers.

Atrazine may suppress hypogastrurid and symphypleonid Collembola in corn field, although they are able to reestablish after several months (Popovici et al., 1977). Apparently, the effect of herbicides on diversity of Collembola is not obvious. Edwards (1968) recognized that, in general, the more active species are more susceptible to herbicides.

Some Aspects of Direct Effects

Field studies have dealt primarily with changes in animal populations. Laboratory studies on species level are very scarce. Those that have demonstrated various aspects of direct effects of herbicides on Collembola.

By exposing Onychiurus quadriocellatus Gisin to 2,4,5-T, Eijsackers (1975) found this herbicide had a lethal effect on Collembola. The higher the dose, the higher the mortality rate. There are two phases in the poisoning process. First is intensification of general activity, and second is lack of activity. At low doses, Collembola began to increase their movements. By increasing the dose, they showed irregular movements of legs or tremor-like contractions. At last, in the highest dose, they became completely immobile. Doses tested were 1.25, 2.5, and 5 cc/m^2.

Further experiments showed that Collembola were able to escape from the contaminated location, but in high dose, most were paralyzed and not able to escape. From these results, since field concentrations are generally low, there is a possibility for Collembola to escape from the contaminated areas. Further, when patchy distribution of herbicide occurs, the mortality will tend to decrease (Eijasackers, 1975).

EXPERIMENT I

Materials and Methods

This study used commercial formulations of Atrazine and Paraquat which are now being applied in till/no till corn fields on the Soil Research Plots, Michigan State University. Two species of Collembola, Folsomia candida (Willem) and Tullbergia granulata Mills, were selected as tested animals. F. candida are laboratory specimens which are widely used in soil arthropods experiments, while T. granulata were collected from an old sod field at the Soil Research Plots. Both species are parthenogenetic and all are females. The soil of collection area is classed as a Spink loamy sand (Whiteside, 1976) and a member of the 4a soil management group (Robertson et al., 1976). The area was sampled, and the samples were analyzed in the Soil Testing Laboratory, Michigan State University. The test results are presented in Appendix VII.

Laboratory rearing techniques basically followed methods described by Snider et al. (1969), utilizing small plastic jars of 27 mm high by 34 mm in diameter. A mixture of plaster-charcoal in an 8:5 ratio was prepared for the rearing substrate. Cultures were maintained at approximately 100 percent relative humidity by dropping distilled water to the substrate daily or as required, with a pipette. Cultures were kept in controlled temperature incubator at 60° F in total darkness.

Eggs of both species were transferred into experimental jars from stock cultures with a needle, one egg into each jar. On the day of hatching, newly hatched Collembola were fed on yeast treated with herbicide. Food was renewed every four days or as required with the same treated yeast to prevent it from being attacked by fungal mycelium.

The treated yeast was prepared as follows: 350 grams powdered brewer's yeast was suspended in 100 ml of a desired concentration of herbicide. The suspension was then filtered, and the filtered yeast was dried at room temperature. Because solubility of Atrazine is limited, there were crystals of Atrazine left on the filter when dried. The crystals and the dried yeast were mixed up together as food treated with Atrazine.

This experiment was run with three concentrations of each herbicide (Paraquat or Atrazine). The concentrations were 600 ppm, 1000 ppm, and 5000 ppm, since the solution which is applied to field for Paraquat is about 600 ppm and for Atrazine is approximately 5000 ppm. Values were calculated from field application suggested by the Cooperative Extension Service, Michigan State University (Chase & Meggit, 1976; Nelson et al., 1976).

A total of 140 individuals of F. candida and 140 individuals of T. granulata were reared individually for this experiment. Observations were conducted daily and terminated after 22 weeks. Table 1 summarizes this experiment.

Table 1.--Experiment I--Number of Treatment Replicates.

Species	Control	Paraquat			Atrazine		
		600 ppm	1000 ppm	5000 ppm	600 ppm	1000 ppm	5000 ppm
F. candida	20	20	20	20	20	20	20
T. granulata	20	20	20	20	20	20	20

RESULTS

Mortality

During the 22 week period, one individual of the controls, F. candida, died at the fifteenth week. Only 3 individuals of F. candida fed on Paraquat died; 2 individuals at 1000 ppm and one individual at 5000 ppm. The same mortalities occurred in feeding Atrazine at 600 ppm and 1000 ppm. However, there were 6 individuals or 30 percent of F. candida fed on 5000 ppm of Atrazine that died during the 22 week period (Table 2).

Mortalities of T. granulata occurred only at 5000 ppm of Paraquat and Atrazine. At 5000 ppm of Paraquat, one individual died, as opposed to 11 individuals at 5000 ppm of Atrazine. As in F. candida, the mortalities began in the early weeks, and those that did not die, survived until the end of the experiment (22 weeks) (Table 3). At 5000 ppm of Atrazine, both species were found to be repelled by food and were rarely discovered feeding. This situation did not happen at lower concentrations of Atrazine and at all concentrations of Paraquat.

Instar Duration

Data presented by Snider (1971) suggests that instar duration can be determined by the presence of exuvia. Exuvia were recorded and then removed. Since F. candida tend to eat their own exuvia (Snider,

Table 2.--Folsomia Candida, Cumulative Percent Mortality, at 60° F.

Week	Control	Paraquat 600 ppm	Paraquat 1000 ppm	Paraquat 5000 ppm	Atrazine 600 ppm	Atrazine 1000 ppm	Atrazine 5000 ppm
6	0	0	0	0	0	0	5
9	0	0	0	0	0	10	10
11	0	0	0	0	0	10	10
12	0	0	0	0	0	10	15
15	5	0	0	0	0	10	30
18	5	0	5	0	0	10	30
21	5	0	10	5	5	10	30
22	5	0	10	5	5	10	30

Table 3.--Tullbergia Granulata, Cumulative Percent Mortality, at 60° F.

Week	Control	Paraquat 600 ppm	Paraquat 1000 ppm	Paraquat 5000 ppm	Atrazine 600 ppm	Atrazine 1000 ppm	Atrazine 5000 ppm
2	0	0	0	0	0	0	15
3	0	0	0	0	0	0	35
4	0	0	0	0	0	0	50
5	0	0	0	0	0	0	55
22	0	0	0	5	0	0	55

1971), daily observations overcame difficulties in finding exuvial remains. However, in F. candida fed on Atrazine, especially at 5000 ppm, the Collembola ate their own exuvia soon after ecdysis. This resulted in only a very small part being left that could have been overlooked. Through careful observations, the remaining part was usually found. Although T. granulata does not eat its own exuvia, identical observational difficulties arose in determining first moults because these Collembola are very small.

Mean instar duration of F. candida and T. granulata are illustrated in Figures 1 and 2. In general, beginning at the sixth instar, the treated F. candida had a longer instar duration than controls. Significant extension of duration was shown by species fed on 5000 ppm of Paraquat and at all concentrations of Atrazine (Appendix I). As compared to lower concentrations, feeding on 5000 ppm of Atrazine had a significantly longer instar duration.

In T. granulata, all treated animals exhibited a longer instar duration than controls (Figure 2), and this was significant at every stadia (Appendix II). As in F. candida, instar duration of T. granulata fed on 5000 ppm of Atrazine was significantly longer when compared to lower concentrations.

Besides high mortalities in both species treated at 5000 ppm of Atrazine, not all of those surviving reached the same instar. Only 6 individuals of F. candida out of 14 individuals surviving for the 22 week period had reached the thirteenth instar. In T. granulata, among 11 individuals surviving, only 10 had reached the seventh instar; 9 reached eighth; 4 reached ninth; and 3 reached tenth. Both species at this treatment concentration were considerably smaller than controls

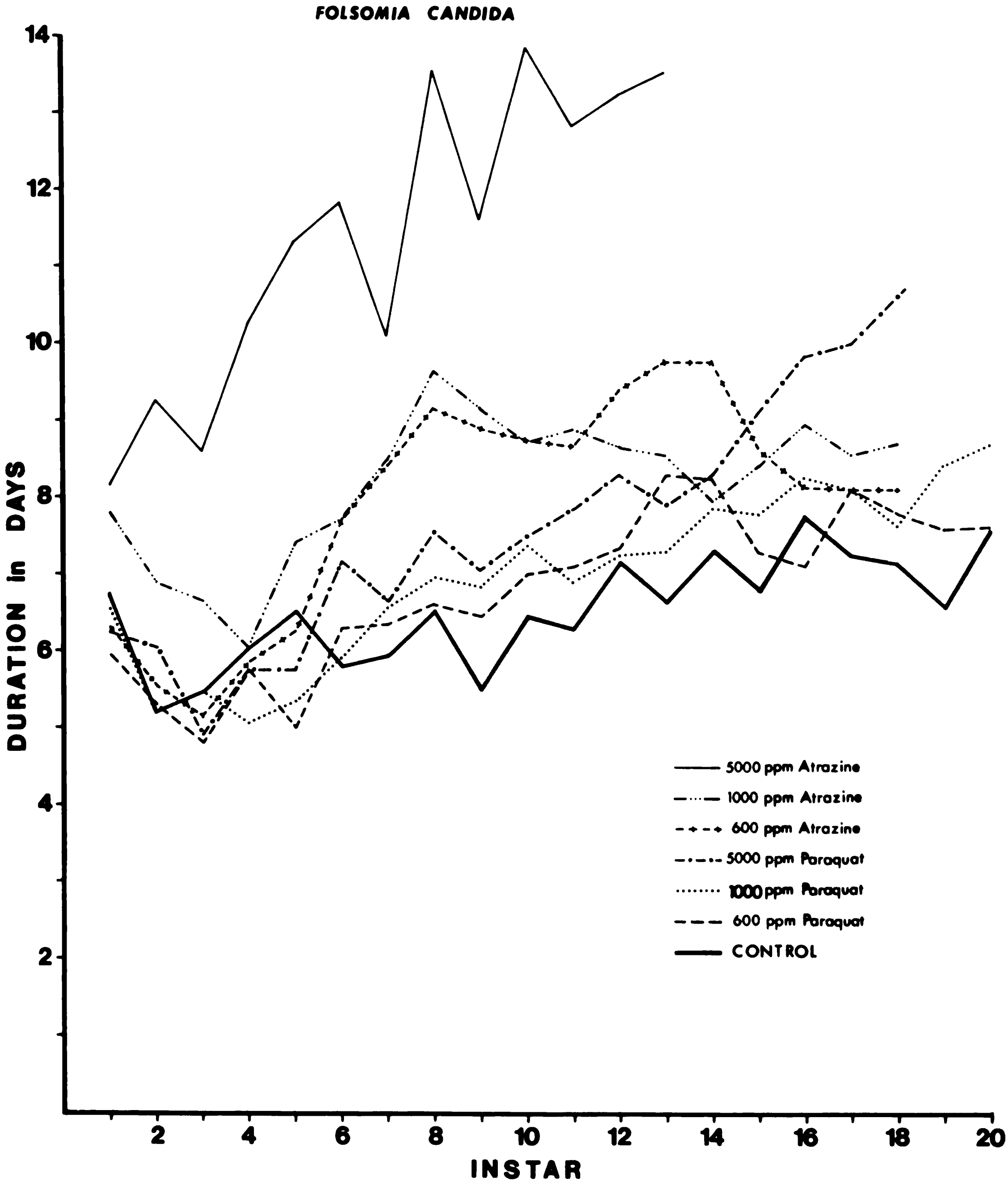

Figure 1. Folsomia Candida, Instar Duration at 60° F.

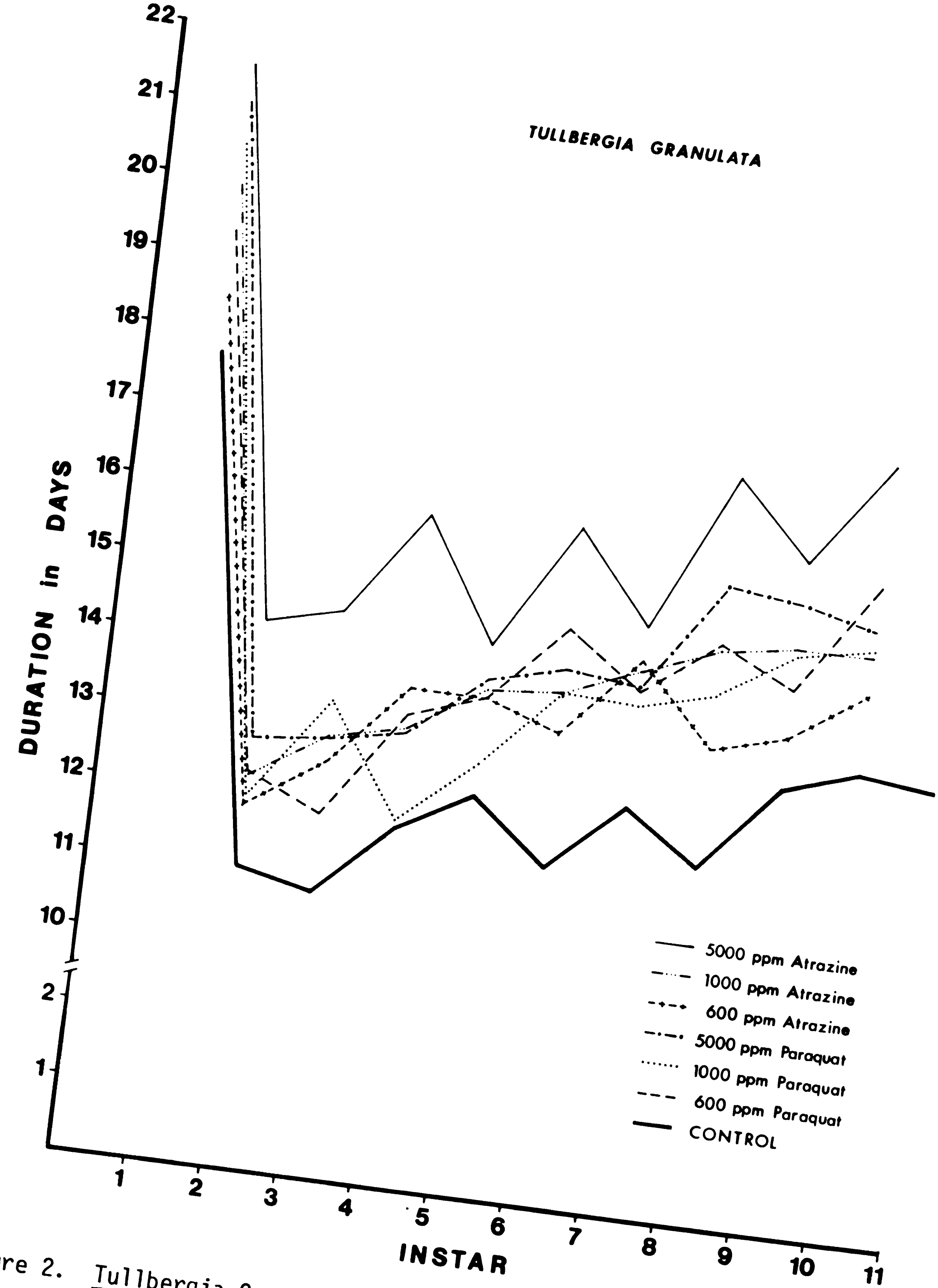

Figure 2. Tullbergia Granulata, Instar Duration at 60° F.

at the same instar. Although there were no measurements taken during the experiment, size differences were obvious.

Egg Production

F. candida begin to lay eggs shortly after the fifth moult is completed and then, more or less regularly, at every two instars (Snider, 1971). In this experiment, almost all the controls laid eggs regularly at every two instars beginning at the sixth instar. Only one individual was found to lay eggs at the seventeenth, nineteethn, and twenty-first, instead of at the sixteenth, eighteenth, and twentieth instar. In treated *F. candida*, irregularities in ovipositions were commonly found, and not all of them began to lay eggs at the sixth instar.

Data in Appendix III and Figure 3 show that the treated *F. candida* significantly reduced their egg production at every oviposition as compared to controls. Feeding on Atrazine also had significantly lower egg production than feeding on Paraquat. Apparently, there was no significant difference between feeding on 600 ppm and 1000 ppm of either Paraquat or Atrazine. Feeding on 5000 ppm of Atrazine inhibited their fecundity. Among them, only 4 individuals were found to lay eggs with less than 4 eggs at every oviposition.

T. granulata begin to lay eggs at the fourth instar, and then at every instar thereafter. As in *F. candida*, the oviposition occurs not more than 24 hours after moulting. Eggs are very small, soft and colorless. As they develop, the eggs increase in size and become more opaque. In contrast to *F. candida*, this species lays eggs singly, not in clumps or clusters. A cluster of 2-3 eggs might be

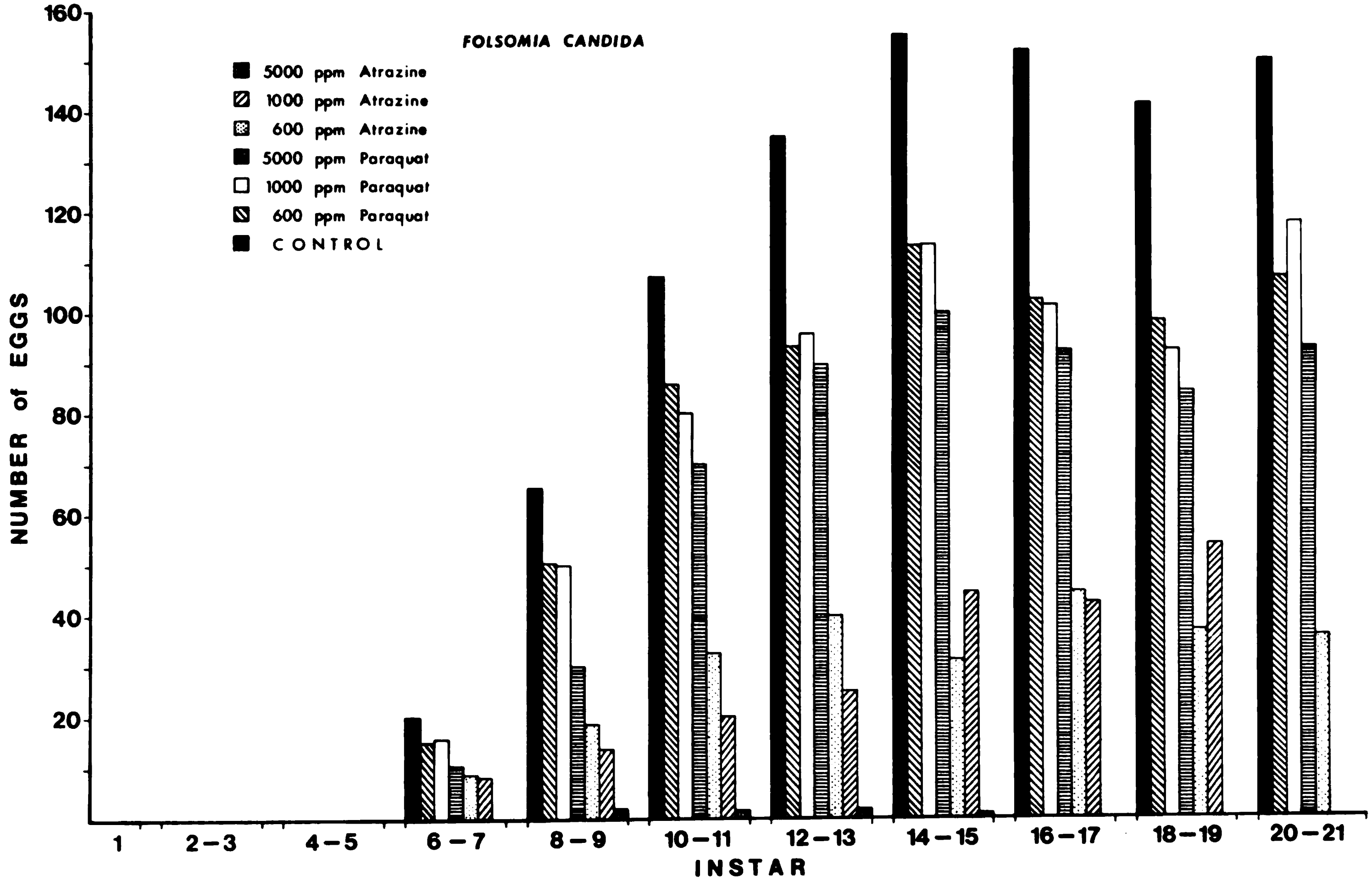

Figure 3. Folsomia Candida, Egg Production at 60° F.

found, but this was uncommon. Laying on wall of jar also happened rarely. Because they are colorless, of small size, and widely dispersed over substrate, observations were made with difficulty.

T. granulata lay few number of eggs. They produce 1-4 eggs at the first oviposition, and then increase numbers in following ovipositions until reaching a peak of 4-11 eggs at eighth to tenth instar. In the following instar, they decrease their egg production (Figure 4 and Appendix IV).

All treated *T. granulata* significantly reduced their egg production as compared to controls. However, there was no difference between feeding on Paraquat and Atrazine. At 5000 ppm of both herbicides, egg productions were significantly different from the lower concentrations. No difference appeared between 600 ppm and 1000 ppm (Appendix IV). Treated *T. granulata* also showed irregularities in ovipositions, and some of them did not begin to lay eggs at the fourth instar.

Egg Viability

For determination of egg viability, only eggs which were laid on the substrate were counted. Eggs laid on wall of jar were counted for egg production and then removed, because these eggs would soon dry. Therefore, these eggs were not included in egg viability determination. After chorionic rupturing, eggs were transferred into fresh jars and were incubated until hatching. Eggs which were attacked by molds were also excluded. Some treated *F. candida* were found to lay "incomplete" eggs, analogous to a mass of jellylike substance, and these were also

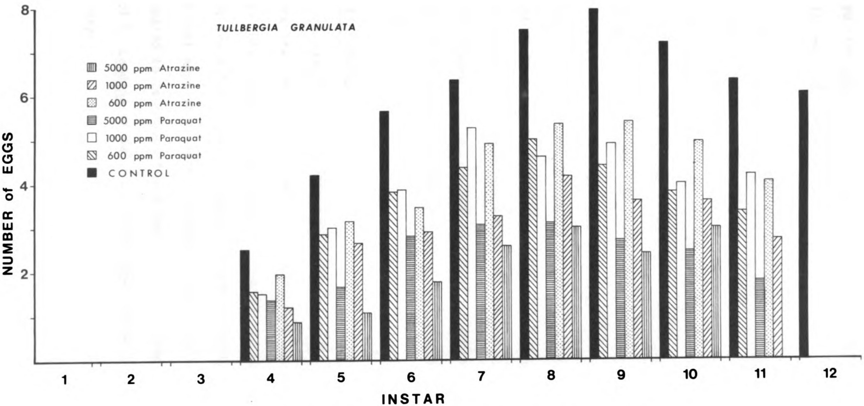

Figure 4. Tullbergia Granulata, Egg Production at 60° F.

excluded from egg production and egg viability determinations. Egg viability was calculated as follows:

$$\frac{\text{total hatching of eggs at same instar}}{\text{total number of eggs at same instar}} \times 100\%$$

Results indicate that, in general, there is no difference between controls and treated Collembola. However, egg viability of *F. candida* fed on 1000 ppm and especially 5000 ppm of Paraquat, by the fifth oviposition, drastically decreased (Table 4). Feeding *T. granulata* on 5000 ppm of Paraquat showed only slightly lower viability as compared to controls (Table 5). There is an indication that high concentrations of Paraquat may affect egg viability. Feeding on Atrazine showed no effect on egg viability. Small egg production at 5000 ppm of Atrazine resulted in high percentage of egg viability (Table 5).

Incubation Period

A number of eggs from every oviposition were taken randomly and were transferred into other jars for recording the incubation period. Days needed for hatching in relation to numbers of eggs in percent are illustrated in Figures 5 and 6. Statistical analysis showed that eggs of treated Collembola had a longer incubation period as compared to controls (Appendices V & VI). Time needed for incubation of *F. candida* at 60° F is 13-22 days, while *T. granulata* needs 25-31 days.

Table 4.--*Folsomia Candida*, Egg Viability (in Percent) per Oviposition, at 60° F.

Oviposition	Control	Paraquat 600 ppm	Paraquat 1000 ppm	Paraquat 5000 ppm	Atrazine 600 ppm	Atrazine 1000 ppm	Atrazine 5000 ppm
1	96.3	96.4	95.3	93.9	100	93.0	--
2	98.3	97.7	97.0	95.0	98.2	98.8	100
3	96.5	95.3	95.1	92.1	99.0	88.7	100
4	89.7	95.1	75.4	90.9	99.0	89.9	100
5	96.8	95.2	73.5	81.3	98.0	78.3	100
6	96.1	96.2	72.5	60.7	95.5	91.7	
7	89.1	89.0	85.9	54.6	89.4	90.0	
8	90.3	89.2	89.0				

Table 5.--*Tullbergia Granulata*, Egg Viability (in Percent) per Oviposition, at 60° F.

Oviposition	Control	Paraquat 600 ppm	Paraquat 1000 ppm	Paraquat 5000 ppm	Atrazine 600 ppm	Atrazine 1000 ppm	Atrazine 5000 ppm
1	100	100	100	85.2	92.3	95.8	100
2	95.2	91.1	95.0	93.9	88.9	94.3	100
3	98.2	98.7	97.4	98.3	89.9	93.1	100
4	96.6	97.8	94.3	92.1	98.0	96.3	100
5	96.4	99.0	95.5	98.8	96.3	95.1	100
6	96.8	96.7	95.9	94.4	97.2	95.8	100
7	95.6	96.1	96.3	95.9	96.9	95.8	100
8	96.7	95.5	95.1	89.7	95.9	94.1	
9	95.9						

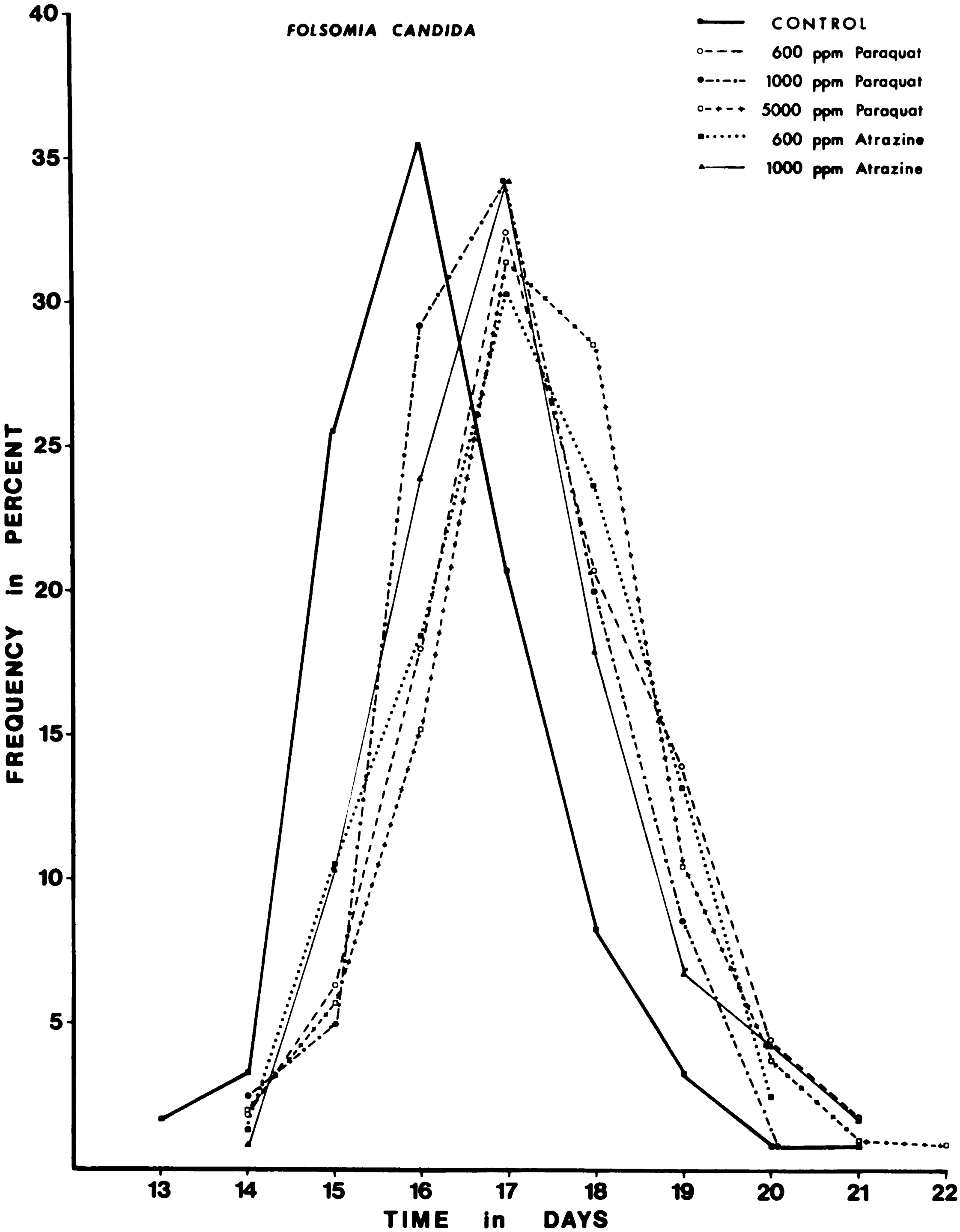

Figure 5. Folsomia Candida, Incubation Period at 60° F. (Relation between number of eggs and time needed for hatching.)

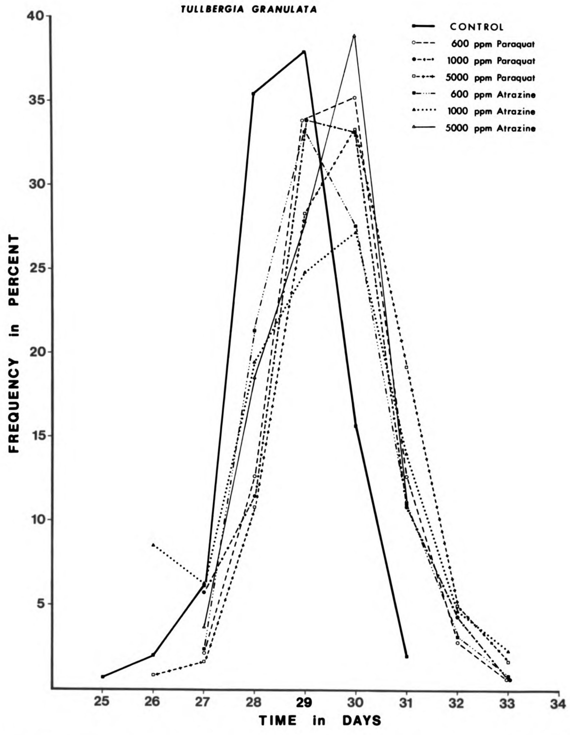

Figure 6. Tullbergia Granulata, Incubation Period at 60° F. (Relation between number of eggs and time needed for hatching.)

DISCUSSION

Observations concerning life history of F. candida as given by Marshall and McE Kevan (1962) and Green (1964) are in some circumstances, different from the present study. Here, rearing methods and food supplies are different from previous studies. However, in many respects, these observations were conducted under conditions as those of Snider (1971) and Snider and Butcher (1973). Therefore, results are practically similar.

Up to the present time, the life history of T. granulata has never been reported. Breeding biology and post-embryonic development of Tullbergia krausbaueri (Börner) were observed by Hale (1965a,b), and later Petersen (1971) found parthenogenesis in the species. Hale's records state that T. krausbaueri begin to lay eggs at the third instar, although only a single specimen was found to do so from among 23 individuals observed. Maximum egg production is reached at the seventh and eighth instars with averages of 7.6 and 7.25. The present study shows that parthenogentic female T. granulata attains sexual maturity at the fourth instar, and maximum egg production at the eighth and tenth instar with means of 7.5, 7.95 and 7.2, respectively (Appendix IV). These data indicate that there is no great variation between the two species. Egg viability of T. granulata is considerably high at every oviposition, while Petersen (1971) reported low viability in parthenogenetic T. krausbaueri due to injuries during transfer from one chamber to another.

Instar duration of T. granulata also has not been specially observed. Observations on T. krausbaueri and other species show that instar duration gradually increases with progressing age (Hale, 1965b). Reports on F. candida state that the first instar to be of longer duration than following immature instars (Green, 1964; Snider, 1971; Snider & Butcher, 1973). The present study clearly shows that first instar of T. granulata is longer than subsequent instars, at least up to the eleventh. First instar is followed by the shortest duration of second and third instar, and then duration increases gradually (Figure 2).

High mortalities were observed in feeding on 5000 ppm of Atrazine. This was not clear whether they were caused by treated food or by starvation. Repellent effects had been observed by Eijsackers (1975) when Onychiurus quadriocellatus Gisin were given a choice between untreated and treated quadrants with 2,4,5-T, a toxic herbicide to Collembola. Obviously, the Collembola were found to choose untreated quadrants. If 5000 ppm of Atrazine has repellent effects, starvation could be the cause of mortality. Therefore, food treated at 5000 ppm of Atrazine may be less palatable to Collembola.

Influence of herbicides on fecundity as well as instar duration has not been recorded. However, modification in fecundity, life span and egg viability in several insects due to sublethal effects of insecticides have been known for several years (Knutson, 1955; Edwards & Thompson, 1973). Although the mechanism is still not understood, apparently reproductive system is affected (Moriarty, 1969). Another explanation, given by Duncan (1963), states that less feeding could cause reduction of fecundity since Aedes aegypti Linn. treated with sublethal dose of dieldrin did not eat as much as the untreated. Here, reduction in feeding was only observed at 5000 ppm of Atrazine, even so it was not observed in other treatments. Besides lessening in fecundity, eggs were also retarded in hatching. In addition, some of the treated F. candida were found to lay "incomplete" eggs as well as reduction of egg viability. Therefore, feeding on herbicides may affect the reproductive system. Further investigations are needed in this area.

Based on argument that herbicides are applied over plants, not to the soil, previous investigators believed that herbicides have no direct effect on soil animals (Fox, 1964; Curry, 1970; Edwards & Thompson, 1973). Accordingly, elimination of weeds could reduce food sources for some soil animals, and indirectly affect soil animal populations. Only Triazine herbicides, including Atrazine, have a direct effect (Edwards, 1970). In addition to the above, foliar-applied herbicides can also penetrate soil by rainfall, although concentrations are considerably lower. Results of the present experiment indicate that herbicides, at least Paraquat and Atrazine, can exert direct effects on Collembola.

Results reported here explain previous studies. Curry (1970) found reduction of collembolan populations in grassland after being sprayed with Paraquat, while Fox (1964) and more recently Popovici et al. (1977) reported the same results after application of Atrazine. Fecundity, instar duration, hatching time, and mortality are among the most important factors in population dynamics. Any distraction to those factors will consequently affect populations. However, in the field, we do not know if the animals actually eat the contaminated food or are able to avoid it.

EXPERIMENT II

Materials and Methods

This experiment was set up to study the possibility of lethal effects of herbicides to Collembola in soil sprayed at applied concentration. Utilizing the same size of jars as in Experiment I, a soil substrate was provided for rearing. The soil was obtained from the Ap horizon of an old sod at the Soil Research Plots, and was dried in the sun for weeks in order to eliminate any animals living in the soil. The soil was described in Experiment I. Before being used as substrate, it was broken up and spread out, then was moistened with distilled water. The moistened soil was then placed into experimental jars until about 1 cm deep. Finally, substrates were sprayed with herbicide at applied concentration, 600 ppm for Paraquat and 5000 ppm for Atrazine (Chase & Meggit, 1976; Nelson et al., 1976). Spray technique utilized a compressed air sprayer with a distance of 50 cm from the surface of substrates.

Fifteen eggs of *F. candida* and *T. granulata* were transferred into each experimental jar, and cultures were kept in a controlled temperature incubator at 60° F in total darkness. When eggs were transferred, those of *F. candida* were one week after being laid, while those of *T. granulata* were two weeks. This experiment had a total of 45 jars for each species and consisted of three experiments.

The number of living animals were counted at three time intervals, 5 jars at each count. First count was conducted in the one week after hatching, and thereafter every week. Counting was made by a simple floatation method, using a saturated solution of sodium chloride. Soil substrate was poured into the saturated solution and stirred. Animals would float and were counted under a binocular microscope. The experimental method is summarized in Table 6.

Table 6.--Experiment II--Number of Treatment Replicates.

Species	Counting	Control	Paraquat	Atrazine
F. candida	I	5	5	5
	II	5	5	5
	III	5	5	5
T. granulata	I	5	5	5
	II	5	5	5
	III	5	5	5

RESULTS AND DISCUSSION

Percent survival of the Collembola observed weekly are presented in Tables 7 and 8. From those tables, it is apparent that Collembola reared on soil sprayed with 5000 ppm of Atrazine suffered lethal effects. There was no significant difference between Collembola reared on soil sprayed with 600 ppm of Paraquat and the unsprayed. Lethal effects of Atrazine significantly began at the second week, and it continued into the following week.

Eijsackers (1975) reported lethal effects on Collembola by exposing O. quadriocellatus to 2,4,5-T. In accordance with Edwards (1970), this study shows that Triazine herbicides have direct effect on soil animals. Since 5000 ppm of Atrazine is the concentration of the solution which is applied over the field, most hazardous effect is on soil animals living in uppermost layers. Those animals have the greatest chance of being in contact with the herbicide. But Eijsackers (1975) suggested that repellent action as previously mentioned permits Collembola to escape from the contaminated area. Ability of animals to escape will tend to decrease mortality under field conditioned. While direct application of Atrazine kills animals, obviously Paraquat exhibits no direct lethal effect.

Table 7.--Folsomia Candida, Percent Survival Reared on Soil, at 60° F.

Week	Control	Sprayed Paraquat 600 ppm	Sprayed Atrazine 5000 ppm	F
1	94.7	92.0	97.3	3.05
	± 5.57	± 2.95	± 3.67	NS
2	97.3	88.0	14.7	256.7**
	± 3.67	± 5.56	± 8.68	$P \leq .001$
3	90.7	88.0	10.7	100.7**
	± 13.8	± 11.95	± 23.8	$P \leq .001$
F	1.10	.59	54.62**	
	NS	NS	$P \leq .001$	

± = standard deviation.

Table 8.--*Tullbergia Granulata*, Percent Survival Reared on Soil, at 60° F.

Week	Control	Sprayed Paraquat 600 ppm	Sprayed Atrazine 5000 ppm	F
1	82.7	77.3	58.7	4.40
	± 3.67	± 12.1	± 19.64	NS
2	86.7	80.0	49.3	9.06*
	± 10.55	± 15.65	± 17.38	$P \leq .05$
3	82.7	80.0	21.34	13.19**
	± 9.91	± 16.33	± 14.64	$P \leq .01$
F	.81	.02	6.33*	
	NS	NS	$P \leq .05$	

± = standard deviation

SUMMARY

1. Fecundity and instar duration of _T. granulata_ are described based on a 22 week period of laboratory observations at 60° F. The species attains sexual maturity at the fourth instar, and maximum egg production at the eighth to tenth, with means of 7.5, 7.95 and 7.2. Duration of the first instar is the longest, followed by the shortest, second and third instars, and then increases gradually thereafter.

2. _F. candida_ and _T. granulata_ were fed herbicides Paraquat and Atrazine at 600 ppm, 1000 ppm, and 5000 ppm. Feeding on herbicides resulted in reduction of fecundity, extension of instar duration and time needed for hatching of their eggs. These results suggest that herbicides affect reproductive system.

3. There was no lethal effect on Collembola reared on soil sprayed with 600 ppm of Paraquat. However, effect was pronounced on Collembola reared on soil sprayed with 5000 ppm of Atrazine.

4. Results of these experiments suggest that Atrazine has the most severe effects on Collembola as opposed to Paraquat.

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APPENDICES

APPENDIX I

Table I.--Folsomia Candida, Instar Duration in Days, at 60° F.

Instar	Control		Paraquat			Atrazine	
		600 ppm	1000 ppm	5000 ppm	600 ppm	1000 ppm	5000 ppm
1	a. 6.75	5.95	6.55	6.25	6.3	7.8*	8.15*
	b. .27	.17	.25	.34	.22	.31	.32
	c. 5-9	4-7	5-9	4-8	5-8	6-10	6-11
2	a. 5.2	5.3	5.2	6.1	5.55	6.9*	9.25**
	b. .16	.18	.22	.17	.23	.40	.31
	c. 4-7	4-6	4-7	5-9	4-7	5-10	6-12
3	a. 5.45	4.8	5.45	4.9	5.15	6.65	8.6**
	b. .28	.16	.31	.16	.18	.48	.53
	c. 4-8	4-6	4-9	4-6	4-6	4-11	3-12
4	a. 6.1	5.75	5.1	5.75	5.85	6.1	10.25**
	b. .50	.12	.13	.10	.11	.39	.59
	c. 3-11	5-7	4-6	5-6	5-7	3-10	6-16
5	a. 6.5	5.0	5.35	5.75	6.25	7.4	11.31**
	b. .36	.18	.21	.27	.24	.28	.51
	c. 4-12	4-6	4-7	4-8	4-8	4-9	8-16

Table I.--Continued.

Instar	Control		Paraquat 600 ppm	Paraquat 1000 ppm	Paraquat 5000 ppm	Atrazine 600 ppm	Atrazine 1000 ppm	Atrazine 5000 ppm
6	a.	5.8	6.3	5.9	7.15	7.7	7.7	11.83**
	b.	.17	.16	.27	.40	.36	.36	1.06
	c.	5-7	4-7	2-7	4-10	5-12	5-12	5-20
7	a.	5.95	6.35	6.55	6.65	8.4*	8.47*	10.11**
	b.	.15	.17	.22	.21	.49	.39	.59
	c.	5-7	5-7	5-9	5-8	5-13	5-11	5-14
8	a.	6.5	6.6	6.95	7.55	9.15*	9.63*	13.53**
	b.	.15	.18	.22	.46	.45	.27	.92
	c.	5-8	5-8	5-8	4-12	6-14	8-12	7-22
9	a.	5.5	6.45	6.85	7.1*	8.9*	9.17*	11.62**
	b.	.15	.20	.22	.36	.44	.51	.60
	c.	4-7	5-8	5-9	4-10	4-12	5-13	8-17
10	a.	6.45	7.0	7.35	7.5	8.74*	8.72*	13.81**
	b.	.18	.28	.27	.27	.42	.29	.86
	c.	5-8	4-9	6-10	5-9	5-13	7-12	7-17

Table I.--Continued.

Instar	Control	Paraquat 600 ppm	Paraquat 1000 ppm	Paraquat 5000 ppm	Atrazine 600 ppm	Atrazine 1000 ppm	Atrazine 5000 ppm
11	a. 6.3	7.1	6.9	7.85*	8.68*	8.89*	12.81**
	b. .22	.35	.32	.25	.43	.39	.61
	c. 4-8	4-10	4-11	5-9	5-12	6-13	10-18
12	a. 7.15	7.35	7.25	8.3	9.4*	8.53*	13.21**
	b. .24	.35	.32	.36	.54	.77	.79
	c. 5-9	4-10	4-11	6-12	5-13	5-17	9-19
13	a. 6.65	8.3	7.3	7.9	9.78*	8.53*	13.5**
	b. .24	.54	.40	.31	.77	.36	.92
	c. 5-9	4-13	4-12	5-12	5-17	6-11	9-15
14	a. 7.3	8.25	7.85	8.3	9.78*	7.94	
	b. .22	.37	.35	.36	.72	.38	
	c. 5-9	6-12	4-10	5-11	4-15	6-12	
15	a. 6.8	7.3	7.78	9.1*	8.64*	8.41*	
	b. .20	.32	.29	.40	.91	.33	
	c. 5-9	4-10	5-10	6-13	4-18	6-11	

Table I.--Continued.

Instar	Control		Paraquat			Atrazine		
			600 ppm	1000 ppm	5000 ppm	600 ppm	1000 ppm	5000 ppm
16	a.	7.74	7.11	8.26	9.85*	8.14	8.94*	
	b.	.22	.34	.30	.58	.59	.35	
	c.	6-9	7-10	6-11	7-16	7-12	6-10	
17	a.	7.26	8.1	8.11	10.0*	8.1	8.56*	
	b.	.20	.46	.27	.50	.59	.44	
	c.	5-8	7-12	5-10	7-14	8-13	5-10	
18	a.	7.16	7.79	7.63	10.7*	8.1*	8.71*	
	b.	.19	.36	.33	.83	.42	.32	
	c.	6-9	7-11	5-10	8-16	8-13	6-11	
19	a.	6.58	7.6	8.42*				
	b.	.22	.51	.42				
	c.	5-8	7-11	5-13				
20	a.	7.58	7.6	8.67*				
	b.	.22	.35	.34				
	c.	6-9	8-13	6-12				

Footnotes for Table I.

a = mean

b = standard error

c = range

* = significantly different from the control, with $P \leq .05$.

** = significantly different from the control as well as the lower concentrations, with $P \leq .05$.

APPENDIX II

Table II.--Tullbergia Granulata, Instar Duration in Days, at 60° F.

Instar		Control	Paraquat 600 ppm	Paraquat 1000 ppm	Paraquat 5000 ppm	Atrazine 600 ppm	Atrazine 1000 ppm	Atrazine 5000 ppm
1	a.	17.7	19.3	20.45	21.0	18.45	20.4	21.5
	b.	.69	1.14	1.01	.56	.72	.65	.55
	c.	15-24	12-29	15-30	18-27	15-24	15-25	15-29
2	a.	11.0	12.25	11.95	12.7*	11.8	12.2	14.25**
	b.	.31	.44	.40	.55	.38	.35	.94
	c.	8-13	8-16	10-16	8-27	10-15	9-15	9-18
3	a.	10.8	11.8	13.3*	12.8	12.5	12.8	14.5**
	b.	.34	.56	.74	.58	.39	.40	.97
	c.	8-14	6-16	9-22	8-18	8-15	9-18	11-23
4	a.	11.75	13.25*	11.85	13.0	13.6*	13.05*	15.92**
	b.	.18	.29	.35	.33	.26	.41	1.27
	c.	10-13	11-16	8-15	9-15	12-16	10-19	12-28
5	a.	12.3	13.6	12.7	13.85*	13.6	13.7*	14.31**
	b.	.31	.36	.40	.35	.26	.35	.38
	c.	10-15	11-18	10-16	10-17	12-16	11-18	12-16

Table II.--Continued.

Instar		Control	Paraquat			Atrazine		
			600 ppm	1000 ppm	5000 ppm	600 ppm	1000 ppm	5000 ppm
6	a.	11.5	14.65*	13.8*	14.1*	13.25*	13.8*	16.00**
	b.	.15	.47	.48	.70	.34	.41	.95
	c.	10-12	11-19	11-20	10-25	11-16	9-16	12-25
7	a.	12.4	13.95*	13.75*	14.0*	14.35*	14.2*	14.8@
	b.	.35	.36	.32	.32	.50	.36	--
	c.	9-17	11-17	10-16	11-17	12-22	12-19	13-17
8	a.	11.75	14.7*	14.0*	15.55**	13.3	14.6*	16.89@
	b.	.32	.40	.40	.60	.31	.32	--
	c.	7-13	12-19	11-19	12-23	10-15	11-17	14-19
9	a.	12.9	14.2	14.65*	15.35*	13.55	14.75*	15.9@
	b.	.20	.46	.33	.46	.25	.41	--
	c.	12-15	11-19	12-18	12-20	12-16	12-18	13-17
10	a.	13.2	15.7*	14.85*	15.11*	14.25	14.75*	17.3@
	b.	.26	.43	.41	.71	.36	.28	--
	c.	12-16	12-20	11-19	10-22	10-17	13-19	15-19

Table II.--Continued.

Instar	Control	Paraquat			Atrazine		
		600 ppm	1000 ppm	5000 ppm	600 ppm	1000 ppm	5000 ppm
11	a. 13.1						
	b. .25						
	c. 11-15						

a = mean

b = standard error

c = range

@ = excluded from statistical analysis because of very low number of observations

* = significantly different from the control, with $P \leq .05$

** = significantly different from the control as well as the lower concentrations, with $P \leq .05$

APPENDIX III

Table III.--<u>Folsomia Candida</u>, Egg Production per Instar, at 60° F.

Instar		Control	Paraquat			Atrazine		
			600 ppm	1000 ppm	5000 ppm	600 ppm	1000 ppm	5000 ppm
1-5		0	0	0	0	0	0	0
6-7	a.	21.45	14.65	16.1	10.95**	8.75*	8.45*	0
	b.	1.30	.80	1.37	.62	1.23	.68	
	c.	12-28	11-22	0-24	5-15	0-17	1-16	
8-9	a.	65.8	50.25*	50.05*	30.35**	18.2*	14.79*	1.5**
	b.	3.26	2.07	2.54	2.42	1.84	1.33	.83
	c.	33-95	36-70	32-71	8-48	0-26	5-25	0-2
10-11	a.	107.6	86.8*	79.9*	69.55**	32.1*	19.5*	1.14**
	b.	4.8	3.5	4.86	3.9	2.74	1.35	.99
	c.	71-142	66-128	42-130	33-105	0-51	8-28	0-3
12-13	a.	135.8	93.75*	96.25*	89.6*	40.05*	26.17*	1.21**
	b.	5.50	4.14	5.54	3.80	3.88	2.5	.80
	c.	62-175	70-128	58-126	59-113	0-61	0-40	0-3

Table III.--Continued.

Instar		Control	Paraquat 600 ppm	Paraquat 1000 ppm	Paraquat 5000 ppm	Atrazine 600 ppm	Atrazine 1000 ppm	Atrazine 5000 ppm
14-15	a.	154.5	103.36*	104.3*	99.8*	30.89*	45.28*	.21@
	b.	6.01	3.88	5.78	6.99	5.25	2.14	--
	c.	85-216	85-138	38-142	12-136	0-69	0-57	0-3
16-17	a.	151.47	102.1*	101.68*	91.79*	43.94*	42.33*	
	b.	6.60	8.54	6.08	9.00	5.17	3.25	
	c.	70-182	0-140	65-149	0-154	0-69	0-57	
18-19	a.	141.21	98.37*	91.84*	83.63*	37.28*	54.23*	
	b.	7.75	10.29	7.54	9.22	3.99	3.94	
	c.	64-204	0-158	0-126	0-133	0-72	0-76	
20-21	a.	150.16	106.67*	118.67*	92.75*	37.75*		
	b.	11.89	13.48	21.61	11.58	2.21		
	c.	0-200	53-139	76-146	24-120	33-42		

a = mean; b = standard error; c = range

@ = excluded from statistical analysis because of very low number of observations

* = significantly different from the control, with $P \leq .05$

** = significantly different from the control as well as the lower concentrations, with $P \leq .05$.

APPENDIX IV

Table IV.--Tullbergia Granulata, Egg Production per Instar, at 60° F.

Instar		Control	Paraquat			Atrazine		
			600 ppm	1000 ppm	5000 ppm	600 ppm	1000 ppm	5000 ppm
1-3		0	0	0	0	0	0	0
4	a.	2.5	1.55	1.5	1.35*	1.95	1.2*	.86**
	b.	.22	.21	.23	.30	.23	.26	.27
	c.	1-4	0-3	0-3	0-3	0-3	0-3	0-2
5	a.	4.2	2.85	3.0	1.65**	3.15	2.65*	1.08**
	b.	.25	.28	.40	.32	.37	.34	.33
	c.	2-6	0-5	0-5	0-5	0-5	0-5	0-2
6	a.	5.65	3.8*	3.85*	2.8**	3.45*	2.9*	1.76**
	b.	.37	.39	.45	.50	.39	.45	.48
	c.	2-8	0-6	0-6	0-6	0-6	0-6	0-3
7	a.	6.35	4.33	5.25	3.05**	4.9	3.25*	2.58**
	b.	.42	.50	.53	.53	.55	.47	.67
	c.	3-9	0-8	0-8	0-7	0-11	0-7	0-3

Table IV.--Continued.

Instar	Control	Paraquat			Atrazine		
		600 ppm	1000 ppm	5000 ppm	600 ppm	1000 ppm	5000 ppm
8	a. 7.5	5.0*	4.6*	3.1**	5.45	4.15*	3.0@
	b. .39	.62	.67	.50	.52	.50	--
	c. 3-11	0-10	0-9	0-8	0-9	0-8	0-4
9	a. 7.95	4.5*	4.9*	2.7**	5.4*	3.6*	2.4@
	b. .28	.68	.62	.50	.66	.54	--
	c. 6-11	0-9	0-9	0-7	0-9	0-8	0-4
10	a. 7.2	3.8*	4.0*	2.45**	4.95*	3.6*	3.0@
	b. .41	.62	.64	.51	.56	.51	--
	c. 4-11	0-10	0-9	0-8	0-9	0-7	0-4
11	a. 6.35	3.35*	4.2*	1.78**	4.05*	2.8*	
	b. .42	.50	.63	.37	.56	.51	
	c. 4-10	0-9	0-8	0-5	0-7	0-8	
12	a. 6.05						
	b. .41						
	c. 2-9						

Footnotes for Table IV.

a = mean

b = standard error

c = range

@ = excluded from statistical analysis because of very low number of observations.

* = significantly different from the control, with $P \leq .05$.

** = significantly different from the control as well as the lower concentrations, with $P \leq .05$.

APPENDIX V

Table V.--Folsomia Candida, Time Required for Hatching in Days at 60° F.

	Control	Paraquat			Atrazine		
		600 ppm	1000 ppm	5000 ppm	600 ppm	1000 ppm	5000 ppm
Mean	16.17	17.34*	16.93*	17.39*	17.14*	17.04*	18.25@
(No. Obs.)	(121)	(111)	(120)	(105)	(76)	(117)	(8)
Std. Dev.	1.3028	1.4045	1.1535	1.3763	1.3136	1.3607	
Range	13-22	14-21	14-20	14-22	14-20	14-21	15-21

@ = excluded from Statistical Analysis because of very low number of observations.

* = significantly different from the controls with $P \leq 0.01$. No significant difference between concentrations within each herbicide.

APPENDIX VI

Table VI.--Tullbergia Granulata, Time Required for Hatching in Days at 60° F.

	Control	Paraquat			Atrazine		
		600 ppm	1000 ppm	5000 ppm	600 ppm	1000 ppm	5000 ppm
Mean	28.63	29.54*	29.48*	29.79	29.34*	29.41*	29.35*
(No. Obs.)	(147)	(142)	(139)	(120)	(127)	(129)	(54)
Std. Dev.	1.0006	1.0690	1.1816	1.1732	1.1834	1.4503	1.0308
Range	25-31	27-33	27-33	27-33	26-33	26-33	27-31

* = significantly different from the controls with $P \leq 0.05$. No significant difference between concentrations within each herbicide.

APPENDIX VII

RESULTS OF SOIL TEST*

Soil pH	:	5.6
Lime index	:	6.4
Lbs. per acre of:		
Phosphorus	:	79
Potassium	:	96
Calcium	:	720
Magnesium	:	177
Percent of total exchangeable bases:		
Potassium	:	4.6
Calcium	:	67.7
Magnesium	:	27.7
Soil texture	:	loamy sand
Manure	:	no
Plowing depth (in.)	:	6

*Recommended Chemical Soil Test Procedure for the North Central Region, North Dakota Agric. Exp. St., North Dakota St. Univ., North Central Pub. No. 221, Bull. No. 499, February 1975.