

Demography of *Paronychiurus kimi* (Lee) (Collembola: Onychiuridae) under the influence of glufosinate-ammonium on plaster charcoal substrate and in artificial soil

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Abstract

The demography of *Paronychiurus kimi* (Lee), a dominant springtail in the paddy fields of Korea, was studied under the influence of glufosinate-ammonium (GA), a herbicide, on two different substrates: plaster-charcoal and artificial soil.

On plaster-charcoal substrate with brewer's yeast provided as food, survival rate of eggs, juveniles and adults, and life table statistics of *P. kimi* did not reveal any significant influence of GA except at an extremely high concentration (1572.70 $\mu\text{g}/\text{cm}^2$). The EC_{50} of GA for reproduction of *P. kimi* was estimated to be 446.21 $\mu\text{g}/\text{cm}^2$. In artificial soil with brewer's yeast, *P. kimi* was susceptible to GA. Reproduction of *P. kimi* was significantly reduced at 0.5 mg/kg and the adult survival rate decreased at 50 mg/kg. The life table statistics showed significant influences of GA even at low concentration, and the EC_{50} was estimated to be 0.039 mg/kg. The fact that acute toxicity of GA to the springtail is negligible does not guarantee negligible impact of the herbicide on springtail populations in the field. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

As primary consumers of organic materials and fungal feeders, collembolans are important biological components of soil ecological systems (Crossley et al., 1992; Seastedt, 1984). Changes in the soil ecosystem caused by toxic herbicides are liable to influence the population dynamics of Collembola directly and also indirectly, by changing the microclimate and depriving them of food.

Herbicides are now used continuously for weed control in crop production and have become ma-

JOR xenobiotics in soil ecological systems. Much work has been done to assess the impact of herbicides on agricultural soils, but with contrasting results. Based on acute toxicity tests, some authors have found the impact on soil fauna of herbicides to be negligible (Fox, 1964), but others have reported the opposite (Eijsackers, 1978a,b; Sabatini et al., 1998). Atrazine and paraquat caused high mortality of *Tullbergia granulata* Mills at 5000 mg/kg and *Folsomia candida* (Willem) at 600 mg/kg (Subagja and Snider, 1981). Atrazine also caused delayed development of *Entomobrya musatica* Stach (Al-Assiuty and Khalil, 1996). However, Rebecchi et al. (2000) suggested that impacts of sulfonylurea on most collembolan species were negligible in the field.

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Assessing the impact of herbicides on collembolan populations based on acute toxicity could be misleading, because the herbicides may not only kill the collembolans directly but also decrease the reproduction potential of survivors. The impact of herbicide on soil ecological systems can be fully understood only by estimating both the acute mortality and demography of the arthropod population under herbicides. Although some demographic studies have been conducted to assess effects of toxicants on soil arthropods (Crommentuijn et al., 1997; Kammenga et al., 1997; Kammenga and Laskowski, 2000), such studies under the influence of herbicides are sparse.

The present study was conducted to evaluate the influence of the herbicide, glufosinate-ammonium (GA), on the death rate and demography of *P. kimi* (Lee), a dominant collembolan in the paddy fields of Korea. Two different substrates were used: plaster-charcoal and artificial soil. The influence of GA on soil ecosystems is currently in dispute (Ahmad and Malloch, 1995; Faber et al., 1997). We tested the impact of GA on the collembolan on the two substrates because toxicity to soil arthropods is seldom compared on different substrates or bases. This could explain the dispute over the impact of herbicides on soil fauna as toxicity of xenobiotics is reported to differ between substrates (e.g. Van Gestel and Ma, 1990).

2. Materials and methods

2.1. Springtails

Paronychiurus kimi, collected from paddy fields in Ichon-Si, Korea in 1996, were maintained in glass jars with a base of moistened plaster of Paris-charcoal substrate, and provided with yeast as food at 20°C in constant darkness at the Population Ecology Laboratory of Korea University.

Hundred *P. kimi* adults, collected randomly from the stock culture, were placed in a glass jar with the base of moistened plaster of Paris-charcoal substrate. They were provided with brewer's yeast and held at 20°C in constant darkness for 7 days. Then the adults were removed and the jars, containing *P. kimi* eggs laid during this period, were held under the same conditions. The resulting juveniles and adults were used in the present studies. Yeast was provided for food.

2.2. Glufosinate-ammonium

GA, or ammonium 4-[hydroxy(methyl)phosphino-nyl]-DL-homoalanine (GA), is made from phosphinothricin, which exhibits strong antibiotic activities (Bayer et al., 1972). GA rarely leaches more than 10–15 cm deep in soil, and it degrades rapidly in non-sterile soil. The dissipation time 50% (DT50) value of GA has been reported to be 1–10 days in sandy loam soil (Gallina and Stephenson, 1992).

A commercial formulation of GA (Basta, Misung Co., Ltd.) containing 18% of the active ingredient GA was used in the present study.

2.3. Artificial soil

Quartz sand, kaolin clay and *Sphagnum* peat (Acadian Peat Moss Ltd.) were mixed in 7:2:1 dry weight ratio. The acidity of the soil was maintained at pH 6 by adding powdered calcium carbonate as necessary. The water content of the soil was maintained at 55% by adding distilled water as necessary (Verhoef and Van Gestel, 1995).

2.4. Demography of *P. kimi* under the influence of GA

To evaluate the egg mortality of the springtail upon exposure to GA, 30 eggs, less than a week after oviposition, were placed in a Petri-dish (90 mm diameter) with a base of plaster of Paris-charcoal (a mixture of plaster-charcoal distilled water in volume ratio 3:1:3). The treated Petri-dishes were sprayed with 2 ml GA solution of 0, 50, 500, 5000, 10,000 or 50,000 µg/ml by using a potter spray tower at 10 psi (Burkard Manufacturing Co.) and held at 20°C in constant darkness. The concentrations corresponded to 0, 1.6, 15.7, 157.3, 314.5, and 1572.7 µg/cm² on the basis of the surface area of the Petri-dish, respectively. Everyday, the number of juveniles hatched was recorded until it was clear that no more eggs would hatch. This experiment was repeated five times per treatment.

To evaluate juvenile mortality, 30 4-week-old juveniles were placed in a Petri-dish with a plaster-charcoal base. The Petri-dish was sprayed with 2 ml of 0, 50, 500, 5000, or 50,000 µg/ml GA solution as described above and held at 20°C in constant darkness. The numbers of living juveniles and adults that had

emerged in each Petri-dish were counted each day until the adults began oviposition. This experiment was repeated five times.

To evaluate adult mortality due to GA, 20 adult springtails less than 1-week-old were placed in a Petri-dish and sprayed with GA solution at the concentration described above. Adult mortality was examined 1 week after treatment, and the treated Petri-dishes were maintained under the same conditions.

The numbers of live adults and eggs laid in each treated Petri-dish were recorded weekly in order to estimate survival and reproduction. The live adults were transferred to fresh Petri-dishes with brewer's yeast as food.

2.5. Effects of GA on demography of *P. kimi* in the artificial soil

About 15 ml of 0, 50, 500, 5000, 10,000 or 50,000 $\mu\text{g/ml}$ GA solution were added to 1500 g of dry artificial soil and mixed thoroughly. The concentrations corresponded to 0, 0.5, 5, 50, 100, and 500 mg/kg on the basis of dry soil weight, respectively. Then 100 g of GA-treated soil, mixed thoroughly with 100 mg of brewer's yeast, was placed in a glass jar (diameter 90 mm, height 90 mm). Into each treated jar, 30 adult, 6-week-old springtails (age including the egg stage), were introduced and left at 20°C in continuous darkness.

Beginning 2 weeks after treatment up to 9 weeks, five jars per treatment were randomly selected each week for destructive sampling, filled with water and gently stirred. The number of springtails floating on the surface of the water was recorded. Life table statistics for each treatment were estimated on the basis of the pooled number of springtails recovered at the observed time.

2.6. Estimation of life table statistics

The intrinsic rate of natural increase (r_m) was estimated from the data using Lotka's equation (see Pielou, 1969)

$$\int_0^{\infty} \exp(-rx) l_x m_x dx \approx \sum_0^{\infty} \exp(-rx) l_x m_x = 1$$

where l_x is the age specific survival rate, m_x age specific fecundity and x the pivotal age (in weeks) of the female. The generation time (GT), finite rate of increase (λ) and net reproduction rate (R_0) were estimated from the equations: $GT \approx \sum x l_x m_x / \sum l_x m_x$, $\lambda = \exp(r)$ and $R_0 = \sum l_x m_x$. The intrinsic rate of natural increase (r_m) was estimated using the maximum likelihood method, and the variance of the life table statistics was estimated using the Jackknife method (Dixon, 1993).

2.7. Data analysis

The median lethal concentration (LC_{50}) (Suter, 1993) of GA for the springtail was determined using the Probit analysis procedure (SAS Institute, 1990) with correction for control mortality (Abbott, 1926). Statistical differences among the values were determined based on 95% confidence intervals of LC_{50} .

To describe changes in the survival rate $S(x)$, the survival data were fitted to the Weibull function (Pinder et al., 1978). This is defined as

$$S(x) = 1 - \exp\left(-\left(\frac{x}{b}\right)^c\right)$$

where x is age, and b and c are parameters of the function (Johnson and Kotz, 1970). Another formula was used to fit cumulative reproduction, which was defined as

$$R(x) = a \left(1 - \exp\left(-\left(\frac{x}{b}\right)^c\right)\right)$$

where a , b and c are parameters.

Survival rates were compared by the Wilcoxon test (Allison, 1995).

Median effective concentrations (EC_{50}) (Suter, 1993) for the intrinsic rate of natural increase (r_m) of the springtail were estimated by fitting the data to the model (Haanstra et al., 1985)

$$r_m = \frac{c}{[1 + \exp\{b(x - a)\}]}$$

where r_m is the intrinsic rate of natural increase, x the natural logarithm of the test concentrations, a the logarithm of EC_{50} , b the slope parameter and c the intrinsic rate of natural increase of the control population.

Influences of GA concentrations on life table statistics were analyzed by ANOVA, and the means were separated using the Fisher least significant difference (LSD) ($\alpha = 0.05$) (SAS Institute, 1990).

3. Results

3.1. Effects of GA on hatching and juvenile survival of *P. kimi*

The effects of GA on egg survival and adult mortality in the springtail were negligible until the GA concentration increased to $314.54 \mu\text{g}/\text{cm}^2$; LC_{50} was estimated as 1966.53 and $941.59 \mu\text{g}/\text{cm}^2$ for eggs and adults, respectively. Juveniles were more susceptible to GA, with LC_{50} estimated at $197.80 \mu\text{g}/\text{cm}^2$. The mortality of the springtail was not significantly higher until a GA concentration of $1572.70 \mu\text{g}/\text{cm}^2$

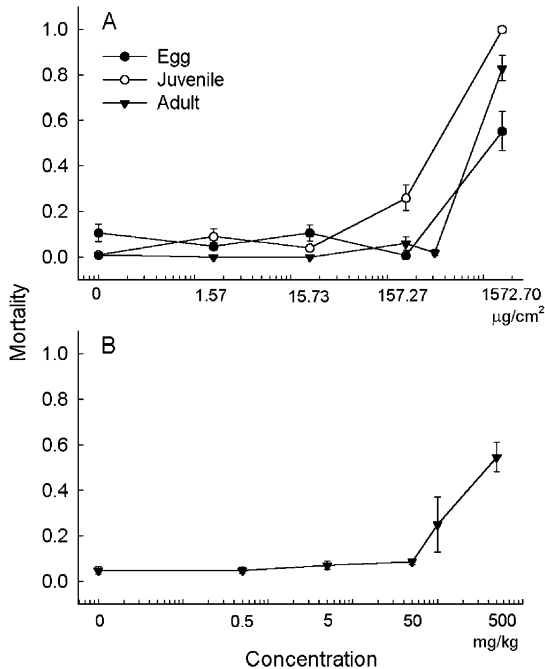


Fig. 1. Egg, juvenile and adult mortality (mean \pm S.E.) of *Paronychiurus kimi* on plaster-charcoal (A) and adult mortality (mean \pm S.E.) in artificial soil (B) treated with different concentrations of glufosinate-ammonium. The number of *P. kimi* observed was 100 and 150 on plaster-charcoal and in artificial soil, respectively.

was reached ($F = 15.62$; d.f. = 4.20; $P < 0.0001$; $\text{LSD} = 0.078$) (Fig. 1).

3.2. Demography of *P. kimi* on plaster charcoal under the influence of GA

The survival rate and cumulative reproduction curve of adult *P. kimi* for different GA concentrations were similar to each other from 0 to $314.54 \mu\text{g}/\text{cm}^2$, but different at $1572.70 \mu\text{g}/\text{cm}^2$ (for survival rate: $\chi^2 = 288.79$, d.f. = 5, $P < 0.0001$; for reproduction: based on 95% CI) (Fig. 2). Estimated life table statistics were also similar, and did not show any influence of GA treatment except that at $1572.70 \mu\text{g}/\text{cm}^2$ (based on 95% CI) (Table 1). The intrinsic rate of natural increase (r_m) was estimated to be in the range of 0.285–0.321 per week. The net reproduction rate (R_0) varied from 47.4 to 79.1 per generation. The EC_{50} for reproduction of *P. kimi* was estimated to be $446.21 \mu\text{g}/\text{cm}^2$.

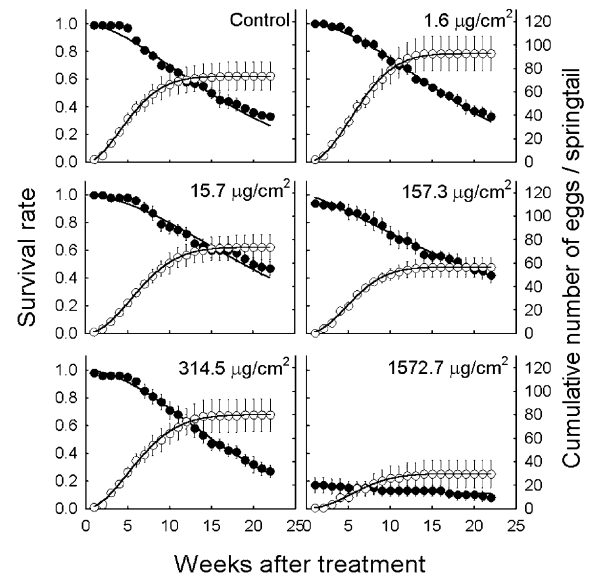


Fig. 2. Survival rate (mean \pm S.E.) (●) and cumulative reproduction curve (mean \pm S.E.) (○) of adult *Paronychiurus kimi* exposed to five different concentrations of glufosinate-ammonium on charcoal-plaster. The number of *P. kimi* observed was 100 at each concentration. The survival rate and the cumulative reproduction were estimated by the functions: $S(x) = (1 - \exp(-(x/b)^c))$ (Pinder et al., 1978) and $R(x) = a(1 - \exp(-(x/b)^c))$ (Johnson and Kotz, 1970), respectively.

Table 1

Life table statistics of *Paronychiurus kimi* treated with different concentrations of glufosinate-ammonium on charcoal-plaster^a

Concentration ($\mu\text{g}/\text{cm}^2$)	r_m	GT	λ	R_0	DT
0	0.32 ± 0.011 a	14.14 ± 0.34 a	1.37 ± 0.015 a	63.23 ± 9.31 ab	2.18 ± 0.074 a
1.57	0.32 ± 0.012 a	14.98 ± 0.27 a	1.38 ± 0.017 a	79.08 ± 14.66 a	2.16 ± 0.086 a
15.73	0.30 ± 0.009 a	15.33 ± 0.35 a	1.35 ± 0.012 a	64.13 ± 10.21 ab	2.32 ± 0.073 a
157.27	0.29 ± 0.008 a	14.62 ± 0.57 a	1.33 ± 0.010 a	47.43 ± 8.53 b	2.43 ± 0.067 a
314.54	0.31 ± 0.012 a	15.04 ± 0.20 a	1.36 ± 0.016 a	66.61 ± 8.18 ab	2.27 ± 0.087 a
1572.72	0.14 ± 0.086 b	15.33 ± 0.30 a	1.14 ± 0.091 b	4.25 ± 3.43 c	– ^b

^a For each parameter point estimates are shown and standard errors estimated using a Jackknife procedure. r_m : Intrinsic rate of natural increase (per week); GT: generation time (weeks); λ : finite rate of increase (per week); R_0 : net reproduction rate; DT: doubling time (weeks). The values with the same letter within a column are not significantly different at the 5% level judged by the LSD test.

^b –: Could not be estimated due to large variance.

3.3. Demography of *P. kimi* on artificial soil under the influence of GA

The effects of GA on the survival rate of *P. kimi* was negligible up to 5 mg/kg, but became significant when the concentration increased further to 500 mg/kg (Fig. 3). However, reproduction of *P. kimi* was strongly

influenced by the GA treatment, even at concentrations as low as 0.5 mg/kg. The cumulative number of progeny produced during the 8 weeks without GA treatment was estimated to be 8.26, but fell to 2.04, 1.67, 0.21 and 0.14 at 0.5, 5, 50 and 100 mg/kg. Adults on the soil treated with 500 mg/kg could not reproduce. The EC_{50} of GA for reproduction was estimated to be 0.039 mg/kg and 95% confidence limits for EC_{50} were 0.0029 to 0.53 mg/kg (Fig. 4).

Life table statistics of *P. kimi* in artificial soil treated with different concentrations of GA are shown in Table 2. The net reproduction rate (R_0) of *P. kimi* without GA treatment was estimated to be 7.53, and at 0.5, 5, 50 and 100 mg/kg was 4.77, 1.22, 0.11 and 0.02, respectively. The intrinsic rate of natural increase (r_m)

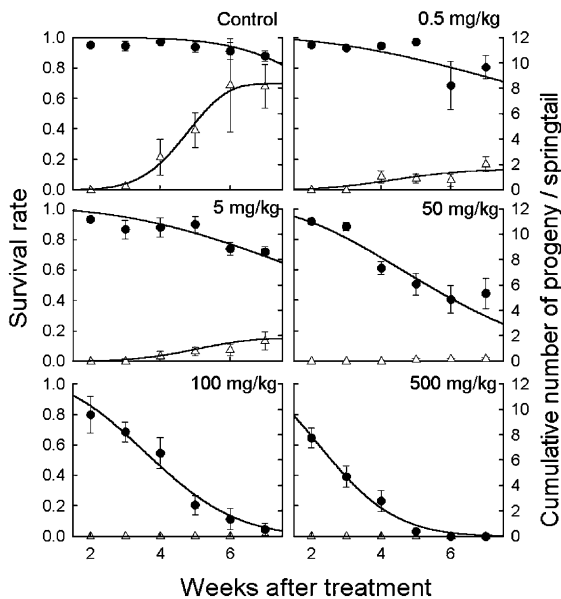


Fig. 3. Survival rate (mean \pm S.E.) (●) of *Paronychiurus kimi* and cumulative progeny (mean \pm S.E.) (△) in artificial soil treated with five different concentrations of glufosinate-ammonium. The number of *P. kimi* observed was 150 at each concentration. The survival rate and the cumulative reproduction were estimated by the functions: $S(x) = (1 - \exp(-(x/b)^c))$ (Pinder et al., 1978) and $R(x) = a(1 - \exp(-(x/b)^c))$ (Johnson and Kotz, 1970), respectively.

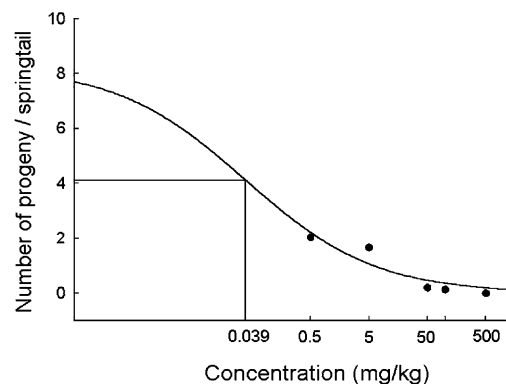


Fig. 4. Dose-response curve for reproduction of *Paronychiurus kimi* in artificial soil treated with five different concentrations of glufosinate-ammonium. The equation is $y = 8.26 / (1 + \exp(0.39(x - 3.25)))$, where y is the number of progeny and x the natural logarithm of the test concentrations.

Table 2

Life table statistics of *Paronychiurus kimi* maintained in artificial soil treated with different concentrations of glufosinate-ammonium^a

Concentration (mg/kg)	r_m	GT	λ	R_o	DT
0	0.18	11.55	1.19	7.53	3.94
0.5	0.11	13.99	1.12	4.77	6.10
5	0.016	12.02	1.02	1.22	42.06
50	-0.19	11.84	0.83	0.11	-
100	-0.33	11.67	0.72	0.018	-
500	- ^b	-	-	-	-

^a r_m : Intrinsic rate of natural increase (per week); GT: generation time (weeks); λ : finite rate of increase (per week); R_o : net reproduction rate; DT: doubling time (weeks).

^b -: Could not be estimated.

without GA treatment was estimated to be 0.176, but for treatments with 0.5 and 5 mg/kg GA it was 0.114 and 0.016, respectively. Further increase in the concentration of GA would cause the *P. kimi* population to decrease.

4. Discussion

The influence of GA treatment on *P. kimi* populations on a plaster-charcoal substrate were negligible. Adult survival curves were similar regardless of the GA concentrations up to 157.27 $\mu\text{g}/\text{cm}^2$, and the EC₅₀ for reproduction of the population was at least ten times higher than the recommended concentration for application in the field (5.4–14.4 $\mu\text{g}/\text{cm}^2$) (Yoon, 1996). However, the *P. kimi* population in artificial soil was significantly affected by the GA treatment, and the EC₅₀ was estimated to be considerably lower than the recommended concentration of GA. Although the survival curves of *P. kimi* in artificial soil under GA treatments were similar to those on the plaster-charcoal substrate, reproduction of *P. kimi* decreased significantly due to the GA in artificial soil compared to plaster-charcoal substrate. This result suggests that GA could suppress *P. kimi* populations in soil by inhibiting reproduction at lower concentrations than recommended for field application, even though GA rapidly degrades in soil (Faber et al., 1997).

The differences in susceptibility of *P. kimi* to GA on the two substrates could be due to physico-chemical properties of the substrates. Acute toxicity of organic

chemicals to collembolans has been reported to be different according to the soil type (Van Gestel and Ma, 1990; Smit and Van Gestel, 1998; Mola et al., 1987; Subagja and Snider, 1981). The life table parameters of springtail populations living on different substrates could also differ. Activated charcoal (activated carbon) is used as an absorbing material for most pesticides, and Booth (1983) has reported that the quantity of charcoal mixed in the plaster of Paris charcoal correlated positively with the number of eggs produced.

The low reproduction of *P. kimi* in artificial soil might be due to the low level of nutrition available to *P. kimi*. The brewer's yeast provided for the springtail was exposed, and thus readily available, on the plaster-charcoal substrate, but in the artificial soil it was mixed and diluted with the soil and could not easily be used as food. Christiansen (1970) and Booth and Andersson (1979) reported a positive relationship between nutrition and reproduction of springtails. Stam et al. (1996) also reported that the life history of *Folsomia candida* changed in response to the food type.

Since the artificial soil is more similar to field soil than plaster-charcoal, the influence of GA on the springtail population in fields should be more reliably inferred from the results in the artificial soil. The failure of the *P. kimi* population to develop in artificial soil treated with GA suggests that the herbicide will have a significant influence on springtail populations in soil and thus on soil arthropod communities. The fact that the acute toxicity toward the springtails is negligible does not guarantee environmental safety of the herbicide. The survival rate curve of *P. kimi* on both substrates were similar, but reproduction of the springtail reduced significantly, retarding population development of the arthropod community.

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