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POPULATION GROWTH RATE AND CARRYING CAPACITY FOR SPRINGTAILS *FOLSOMIA CANDIDA* EXPOSED TO IVERMECTIN

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Abstract. Forecasting the effects of stressors on the dynamics of natural populations requires assessment of the joint effects of a stressor and population density on the population response. The effects can be depicted as a contour map in which the population response, here assessed by population growth rate, varies with stress and density in the same way that the height of land above sea level varies with latitude and longitude. We present the first complete map of this type using as our model *Folsomia candida* exposed to five different concentrations of the widespread anthelmintic veterinary medicine ivermectin in replicated microcosm experiments lasting 49 days. The concentrations of ivermectin in yeast were 0.0, 6.8, 28.8, 66.4, and 210.0 mg/L wet weight. Increasing density and chemical concentration both significantly reduced the population growth rate of *Folsomia candida*, in part through effects on food consumption and fecundity. The interaction between density and ivermectin concentration was "less-than-additive," implying that at high density populations were able to compensate for the effects of the chemical. This result demonstrates that regulatory protocols carried out at low density (as in most past experiments) may seriously overestimate effects in the field, where densities are locally high and populations are resource limited (e.g., in feces of livestock treated with ivermectin).

Key words: *Collembola; density dependence;* Folsomia candida; *food availability; microarthropod; soil; soil invertebrate; springtail.*

Introduction

Forecasting the effects of toxicants on the dynamics of natural populations is a major aim of ecotoxicology (Moe et al. 2001). This requires assessment of the joint effects of a toxicant and population density on the population response. In current practice, in contrast, the majority of chemical risk assessments use findings from individual-level endpoints (Herbert et al. 2004). Population growth rate (pgr) has been identified as the best measure of how a population responds to a chemical (Van Straalen et al. 1989, Forbes and Calow 1999), and has been widely measured in life table response experiments (LTREs; Caswell 1996, Levin et al. 1996), but these studies have generally been performed at low population densities and/or high food levels (Kammenga et al. 1996, Levin et al. 1996, Sibly 1996, Forbes and Calow 1999, Hansen et al. 1999) and so have not incorporated the effects of density on pgr.

Previous studies that have investigated interactions between population density and toxicants have not given uniform results (Forbes et al. 2003). Thus invertebrate microcosm studies involving a variety of stressors have produced examples in which the interactive effects on pgr of density and a stressor were more than additive (e.g., Cecchine and Snell 1999), less than additive (e.g., Sibly et al. 2000), or additive (e.g., Kluttgen and Ratte 1994). Differences between the types and form of interaction from these studies could be attributed to variations in experimental design, in particular to whether populations were increasing exponentially or declining (Forbes and Calow 1999, Forbes et al. 2001, 2003). These studies, however, did not characterize the effects of density in the region of carrying capacity (Forbes et al. 2003). As natural populations may often occur around carrying capacity (zero growth rate), changes in carrying capacity with stress levels are of ecological importance (Sibly 1999). Working in the region of carrying capacity, Barata et al. (2002) showed for a cladoceran, and Forbes et al. (2003) showed for polychaete populations, that the effects of a toxicant were significantly reduced in the region of carrying capacity.

The anthelmintic veterinary medicine ivermectin was chosen as the chemical stressor in the experiments reported here. Ivermectin was introduced as a broad-spectrum antiparasite medication in the mid-1980s. Like some other veterinary medicines, ivermectin is poorly absorbed by the gut of the treated animal and is excreted largely unmetabolized in the feces or urine of treated animals, thus causing potential environmental risk (Chiu et al. 1990). Ivermectin causes paralysis or numerous sublethal metabolic disorders and finally death of invertebrates that ingest or absorb it via the cuticle (Boxall et al. 2004). The toxicity, behavior, and fate of

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PLATE 1. *Folsomia candida*, a "standard" soil arthropod. Photo credit: © S. Hopkin, used by permission.

ivermectin have been relatively well studied in the field (e.g., Bull et al. 1984, Sommer and Overgaard Nielsen 1992, Cook et al. 1996). By inhibiting dung fauna, ivermectin reduces the rate of degradation of dung and so slows the return of vital nutrients such as nitrogen to the soil (Boxall et al. 2004). The effect of ivermectin on dung fauna has been well documented although there is a shortage of information on its effects on non-target soil-dwelling invertebrates (Madsen et al. 1998, Jensen et al. 2003). Ivermectin is very toxic to the springtail *Folsomia fimetaria*. When the effects of ivermectin (exposed via soil pore water) were assessed using adult survival and reproduction as endpoints on the springtail *F. fimetaria*, it was found that reproduction was the most sensitive endpoint to ivermectin with an EC_{10} value of 0.26 mg/kg dry soil; the EC_{50} value was 1.7 mg/kg dry soil (Jensen et al. 2003).

Collembola are found at high population densities in manure and sewage sludge (Jensen et al. 2003). They play an important role in decomposition processes and in forming soil microstructure (Rusek 1998, Cragg and Bardgett 2001). The widespread and abundant species *Folsomia candida* (see Plate 1) is the most extensively studied in terms of life history and is employed in the International Organisation for Standardisation (1999) reproduction test (see review by Fountain and Hopkin 2005). Many studies have employed *F. candida* as an indicator of potential toxicity of inorganic and organic chemicals that occur in sewage sludge or manure (dosed with agrochemicals) deposited onto arable land (Fava and Bertin 1999, Cole et al. 2001, Diez et al. 2001, Gejlsbierg et al. 2001, Crouau et al. 2002, Lock and Janssen 2002*a, b, c*, Frische 2003, Kuperman et al. 2004).

The intention of the work presented here is to determine the combined effects of population density and chemical stress on pgr and carrying capacity of a key indicator species in ecotoxicology, *F. candida,* under controlled laboratory conditions. From the results of Barata et al. (2002) for cladocerans and Forbes et al. (2003) for polychaetes we hypothesized that high density *F. candida* populations exposed to ivermectin might compensate for the initial mortalities caused by the toxicant, reducing the effects of ivermectin in the region of carrying capacity.

MATERIALS AND METHODS

Life cycle of Folsomia candida

At a constant temperature of 20°C, laboratory populations of the parthenogenetic species *F. candida* live on average for 140 days, growing indeterminately, and passing through as many as 38 instars (Snider and Butcher 1973). Oviposition occurs in alternate instars every seven to 10 days starting from about the seventh

instar (~20 days in age). During early instars, about 20 eggs are laid in each oviposition but this increases to ~100 per batch at around the 20th instar before reducing to ~60 at the 30th instar (Snider 1973). Eggs usually take between 12 and 13 days to hatch at 20°C.

Experimental animals

All experiments were conducted with the "Reading strain" of *F. candida*. These are all descendants of a single female selected in 1994 from a laboratory culture obtained from the University of Southampton, UK. The culture substrate was a moistened layer (1 cm deep) of an 8:1:7 mixture of plaster of Paris:graphite powder:distilled water, by weight, in clear plastic boxes (6 cm high × 17 cm long × 11.5 cm wide). The *F. candida* densities were kept at ~300 individuals per culture box. Animals were removed when densities became too high. Food, dried baker's yeast (*Saccharomyces cerevisiae*) suspended in distilled water, was always available in excess. To maintain the humidity within the boxes at 100%, the underside of the lids of the boxes were sprayed every 48 hours with distilled water. Synchronous cultures of 14 ± 1 day old *F. candida* were established in plastic boxes with a moist layer of plaster of Paris and graphite powder in line with International Organisation for Standardisation (1999) and Wiles and Krogh (1998) culturing systems. To each container, 100 adults from the stock culture were added. Food (baker's yeast) was always available in excess. Three days after adults had been introduced into the culture containers they were removed. The eggs laid during this period were allowed to hatch and develop for 14 days before transfer to the experimental containers.

Experimental environment

The experiment was carried out in a temperature-controlled room maintained at 20 ± 1°C. Photoperiod was controlled to provide 24 hours of light. The light intensity directly above the experimental systems was kept constant. Each population was contained in a 60 ml plastic container (6 cm high × 3.7 cm in diameter) with a plastic screw-top lid (Bibby Sterilin [now Barloworld Scientific], Essex, UK). The culturing substrate mixture was made up in small quantities (80 g plaster of Paris: 10 g graphite powder: 70 ml distilled water) and poured into the containers to provide a layer 0.5 cm in depth. The mixture was left for at least two days to set and the test containers were thoroughly washed and soaked with distilled water before use. Excess water was removed and one glass coverslip of 13 mm in diameter was placed in the center of the surface of the substrate of each container. The methodology of this technique is described in greater detail by Fountain and Hopkin (2001).

Experimental design

Synchronous laboratory populations of 14 ± 1 day old juvenile *F. candida* were initiated at five different densities determined by food consumption rates in a pilot experiment (Noël 2004). The densities chosen were 0.19, 0.37, 0.74, 1.49, and 2.98 juveniles per cm^2 floor area, corresponding to 2, 4, 8, 16, and 32 juveniles per microcosm. Each density was exposed to a solvent control and four concentrations of ivermectin via the food. Treatments were selected to provide nominal concentrations of 0, 3, 10, 30, and 100 μg/g of yeast solution (wet weight). For each density and chemical treatment there were four replicates, giving one hundred test populations in total. All of the populations were fed a fixed ration of food (5 μl of yeast solution consisting of 1 g of dried baker's yeast in 8 ml of double distilled water, spiked as required with ivermectin) placed on a coverslip in the center of the microcosm. This was replenished weekly.

Test chemical

Ivermectin was obtained from Sigma, UK (93.9% ivermectin B_{1a}). For each of the five chemical treatments (control, low, low medium, medium, and high), a test solution was prepared using known volumes of acetone and ivermectin to give the required nominal concentration per microcosm. The actual concentrations of ivermectin in stock solutions were measured using High Performance Liquid Chromatography (HPLC). For each concentration, 1 ml samples were placed into centrifuge tubes using a micropipette. All tubes were centrifuged (MSE Centaur 2; SANYO Biomedical Europe BV, Loughborough, Leicestershire, UK) at 2200 m/s^2 for 10 min. The supernatant was removed and placed in analysis vials. The extraction solution, 2 ml of methanol and 1 ml of McIlvaine pH 4 buffer preparation (154 ml of 0.2 mol/L citric acid + 96 ml 0.4 mol/L Na_2HPO_4 + 250 ml distilled water) was added to each tube and vortexed (Yellowline TTS 2; IKA, Staufen, Germany) to resuspend the yeast pellet. The centrifuge tubes were placed in a sonic bath (Ultrawave U300; Wolf Laboratories, Pocklington, York, UK) for 10 min and then centrifuged at 2200 m/s^2 for 10 min. The extraction solution was removed and placed in an analysis vial. The extraction process was repeated once more and the extraction solution was removed and placed in an analysis vial. Contents of the analysis vials were then analyzed using HPLC. The residual yeast pellet was resuspended using 1 ml of methanol for each centrifuge tube, the supernatant and pellet were decanted onto a filter paper and dried in the oven at 105°C. The dried yeast was weighed to the nearest μg.

HPLC analysis was performed using a Dionex Summit HPLC system (Dionex, Idstein, Germany) comprising a GINA 50 autosampler and a P580 quaternary gradient pump with a UVD 170S UV/visible spectrophotometric detector that allowed four wavelengths to be simultaneously monitored. This system was controlled by Chromeleon software. Separations were performed on a 150 cm × 4.6 mm 4 μm GENESIS C18 column (Kinesis Solutions, Bedfordshire, UK). Mobile

phase used was 94% acetonitrile and 6% water with a flow rate of 1 ml/min. Detection was done at 245 nm. Time to elution was about 11 minutes. Calibration standards 0, 0.01, 0.1, 1, 10, and 100 μg/ml were used. The actual concentrations of ivermectin in yeast were measured at 0.0, 6.8, 28.8, 66.4, and 210.0 mg/L wet weight.

Biological parameters and analyses

Automated image analysis was employed to measure the cross-sectional areas of the individuals in the population once a week for seven weeks. The image analysis equipment consisted of a color video camera (JVC, 3CCD, RGB, equipped with a 12.5–75 mm zoom lens and polarizing filter to reduce light reflections) connected to a frame grabber (Matrox Meteor; Matrox Video and Imaging Technology Europe, Buckinghamshire, UK) with a fiber optic cold light source (Schott KL1500, Galvoptics, Essex, UK) to illuminate the specimens. The overall effect maximized the contrast between background and animals. The image was focused manually via a monitor connected to the frame grabber. Image processing and image analysis was performed using a customized program developed by KS 300 Imaging System, Release 3.0 (Carl Zeiss Vision GmbH, Haubergmoos, Germany).

For each population, the glass coverslip was removed and 1 cm^3 of distilled water was poured into the test container so that the animals floated. *Folsomia candida* are capable of moving on the surface of the water, by using the first two pairs of legs for locomotion and the last pair for balance. The surface tension of the water caused the animals to move towards the center of the vessel, thus appearing in frame and isolated from reflections of the sidewalls of the container. The microcosm was then photographed. The captured image was initially enhanced to increase contrast and was subsequently segmented to separate specific features of the image. The software produced a binary image, and calculated the size of each individual. Size (measured in mm^2) was defined as the area within the outline of the live animal (not including antenna) seen from above. "Population area" was the sum of the sizes of the individuals in the microcosm. Only objects above a threshold of 0.01 mm^2 were processed. This excluded impurities and reflections from the background but still included small juveniles. The image processing stages were recorded in a macro program and data collection was thereby automated. The accuracy of the analytical software was checked by manually measuring a number of randomly selected images. Although individuals were occasionally obscured by other individuals, when the area occupied by individuals was plotted against their number, the relationship was found to be linear. Since population growth rate (pgr) is derived from the ratio of two estimates of population size, estimates of pgr should therefore be unbiased.

Population growth rate for each population was calculated as $1/6 \log_e(A_7/A_1)$ for densities from 2 to 32, where A_t represents population area in week t. Our pilot experiment seemed to suggest that this range of densities would result in values close to pgr = 0 for all ivermectin concentrations, but final experimental results showed that the densities were not always high enough (see Fig. 1). However in the later weeks of the experiment density increased, providing additional information, and this was used in preparing Fig. 3 to obtain estimates of pgr for "initial densities" 64 and 128 from regressions of $\log_e(A_{t+1}/A_t)$ against $\log_e(A_t)$.

After image analysis, food was replenished and the lid of each container was sprayed with distilled water. To give a general indication of food consumption per microcosm, we recorded simply whether or not the food had been completely consumed, as food could not be accurately weighed due to moisture fluctuations and very small quantities. The number of eggs present was determined using an Olympus MVZ microscope (Olympus, Japan) and a hand counter. Fecundity was measured in this way each week for four weeks. The combined effects of density and chemical stress on fecundity and pgr were analyzed using a two-way ANOVA (Minitab Release 13.1).

Results

Trajectories of population size over time are shown in Fig. 1. Under control conditions (no ivermectin) populations initially grew exponentially. Similar patterns of population growth were seen in the other treatments, but as ivermectin concentration increased, initial growth rates decreased. In order to analyze these processes statistically we used population growth rate (pgr) as an index of the population response. If pgr is constant and positive, the population grows exponentially, if pgr < 0 the population declines, and at carrying capacity, pgr $= 0$.

The effects of ivermectin and initial density on pgr are shown as line graphs in two forms in Fig. 2. Pgr declined with ivermectin ($F_{4,75} = 97.25$ $P < 0.001$) and initial density ($F_{4,75} = 56.8$, $P < 0.001$) and there was an interaction between the effects of concentration and initial density ($F_{4,75} = 3.76$, $P < 0.001$) such that ivermectin caused a greater decline in pgr at lower initial densities. It should be noted that the declines in pgr with ivermectin concentration appear sharper because ivermectin concentration is plotted on a log scale.

The joint effects of initial density and ivermectin on *F. candida* can be seen more clearly by plotting contours of population growth rate, as in Fig. 3, which shows all the key features of our results. Population growth was fastest at low initial density and low ivermectin concentration. As initial density and ivermectin concentration increased, pgr decreased. Carrying capacity (equilibrium density) occurs at the pgr = 0 contour. The position of the pgr = 0 contour indicates that carrying capacity remains similar over a range of

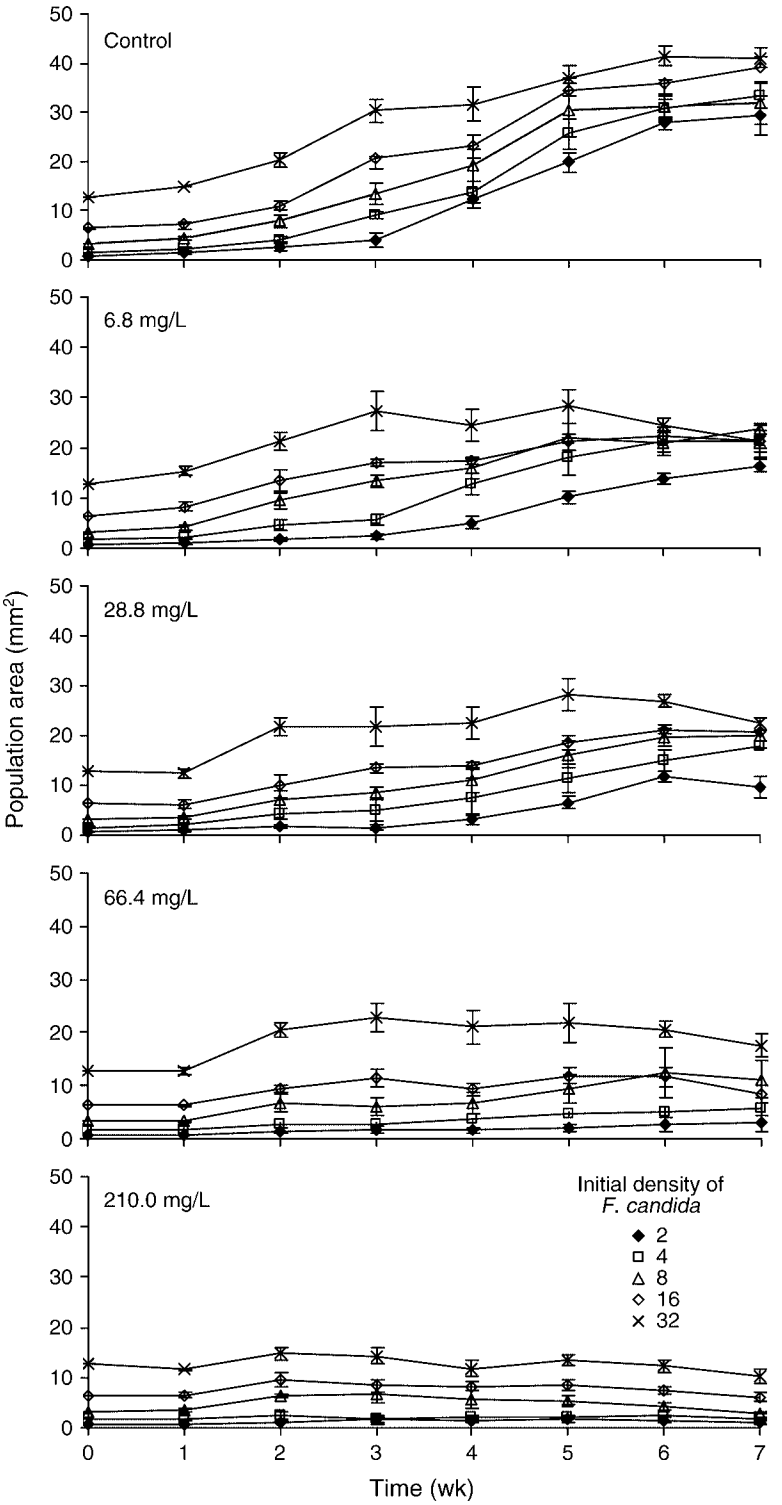

FIG. 1. Population area (mean ± SE, $n = 4$ replicates) in each of the seven weeks of the experiment. Each panel relates to one concentration of ivermectin as indicated in the panel label. Lines and symbols refer to initial densities of *F. candida*.

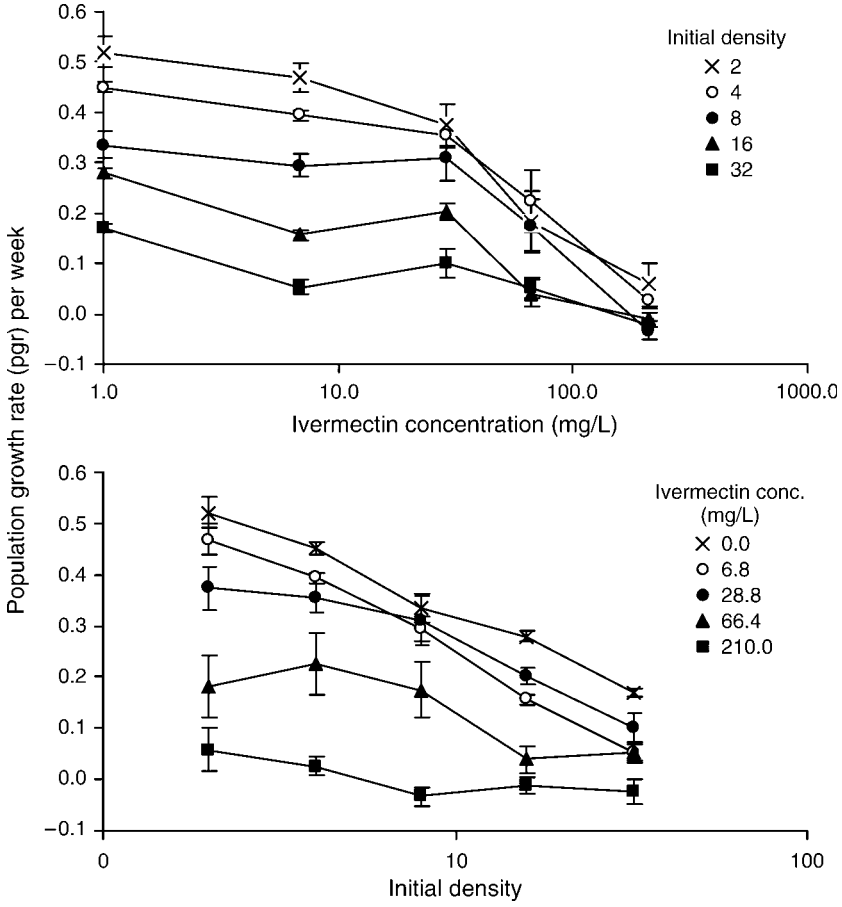

FIG. 2. The effects of ivermectin and density of *F. candida* on population growth rate (pgr; mean ± SE, $n = 4$) shown as line graphs in two forms. (a) Mean pgr per week in *F. candida* exposed to concentrations of ivermectin at different initial densities (log scale). For convenience, ivermectin concentration zero has been represented as 1 on the *x*-axis. (b) Data as in (a), but plotted against initial density (log scale), with symbols representing ivermectin concentrations.

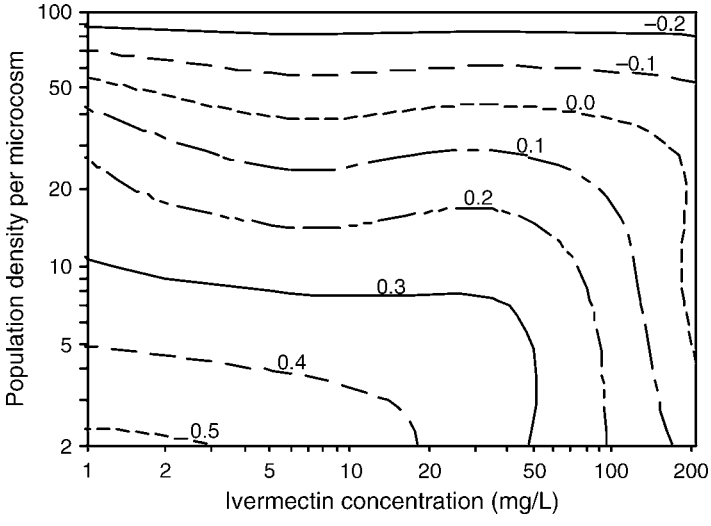

FIG. 3. Joint effects of initial population density and ivermectin concentration (log–log scale) on population growth rate (pgr) represented in a contour map in which height represents pgr. Numbers indicate the pgr of a given contour. Microcosm floor area was 10.75 cm^2. Contours were fitted using Minitab Release 13.1. Ivermectin concentration zero has been represented as 1 on the *x*-axis as in Fig. 2.

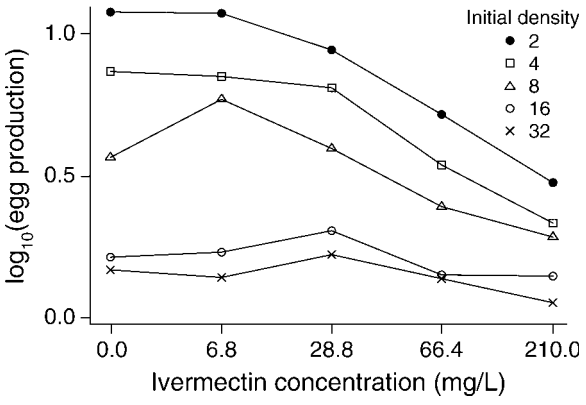

FIG. 4. Mean fecundity, $\log(x+1)$, per week averaged over the first four weeks in which *F. candida* populations were exposed to ivermectin at different initial densities. The pooled standard deviation was 0.324.

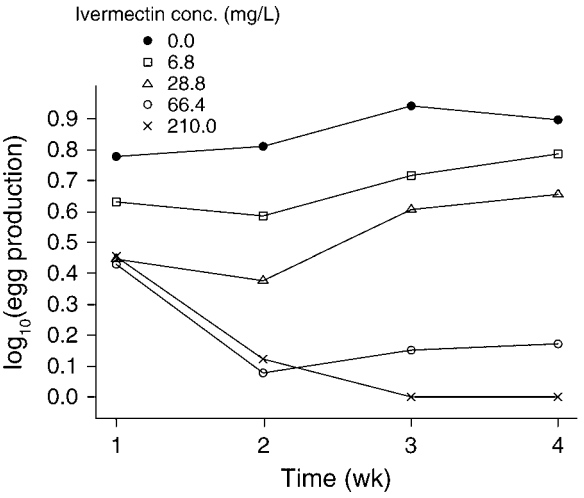

FIG. 6. Mean fecundity, $\log(x+1)$, per week in *F. candida* populations exposed to ivermectin. The pooled standard deviation was 0.340.

ivermectin concentrations until a concentration of 150 mg/L, above which carrying capacity declines. The effect of the interaction between initial density and ivermectin can be seen by comparing cross sections through the surface at low and high densities. Along a transect through Fig. 3 at low initial density, pgr declines with chemical concentration, whereas at high density the chemical has no effect on pgr.

To help in the interpretation of these results some supporting information regarding fecundity and food supply is presented in Figs. 4–6. Fecundity estimates were feasible in the first four weeks of the experiment, and these are presented in relation to chemical concentration and initial density in Fig. 4. Fecundity declined with increasing initial density ($F_{4,300} = 21.89$, $P < 0.001$), and declined overall with ivermectin concentration ($F_{4,300} = 86.02$, $P < 0.001$). As with pgr there was an interaction between the effects of concentration and initial density ($F_{16,300} = 2.24$, $P < 0.01$) such that at high density there was little change in fecundity as concentration increased (Fig. 4).

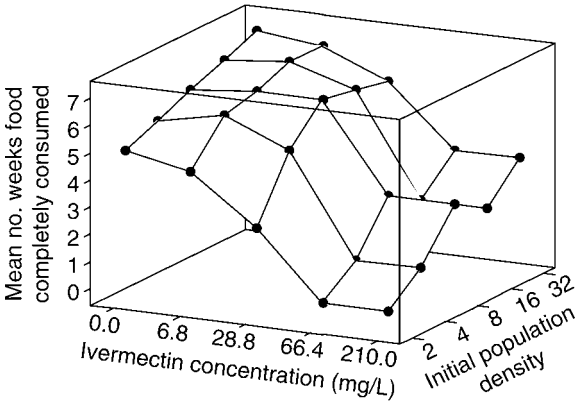

FIG. 5. Effects of ivermectin concentration and initial population density on food consumption for the seven weeks of the experiment. The vertical axis represents the number of weeks that the food ration in the microcosms was completely consumed. The pooled standard deviation was 1.083

While it was not feasible to make precise measurements of food consumption, some assessment was possible by recording at the end of each week whether or not the previous week's food ration had been completely consumed. The relationships between food consumption, initial density, and ivermectin concentration are shown in Fig. 5. At all concentrations food consumption increased when there were more springtails ($F_{4,75} = 20.74$, $P < 0.001$). Food consumption declined as ivermectin concentration increased ($F_{4,75} = 71.56$, $P < 0.001$). There was no interaction between the effects of initial density and chemical concentration ($F_{16,75} = 0.75$, not significant).

The effects of ivermectin on fecundity changed with time in a way that depended on concentration ($F_{12,300} = 4.64$, $P < 0.001$). Fecundity declined with time at high ivermectin concentrations, but increased with time at low concentrations (Fig. 6).

DISCUSSION

The aim of this study was to determine the combined effects of population density and ivermectin on food consumption, fecundity, and population growth rate (pgr). Overall we found that both density and ivermectin had negative effects on all three response variables. The best overview of the population responses is provided by the contour plot in Fig. 3. This shows that there is an interaction between the effects of density and ivermectin. These effects are 'less-than-additive' in the terminology of Forbes et al. (2001). Taking a transect at low density, pgr declines with chemical concentration, whereas at high density the chemical has no effect on pgr. Thus at high density the population is able to compensate for the effects of the chemical. Similar results were found in the

only two comparable studies that recorded the effects of a stressor in the region of carrying capacity (i.e., Forbes et al. [2003] on polychaetes and Barata et al. [2002] on the tropical cladoceran *Moinodaphnia macleayi* exposed to cadmium). The finding that resource limited or high density populations buffer toxicant effects has also been described by Sibly (1996), Calow et al. (1997), Grant (1998), and Forbes et al. (2001). In our study, the mechanism responsible must involve fecundity, since the fecundity effects shown in Fig. 4 mirror those of Fig. 3. It is indeed possible that all the effects of density and ivermectin on population growth rate shown in Figs. 2 and 3 are a result of their effects on fecundity. A likely mechanism is that the chemical produces adult mortality, as a result of which more food is available for the survivors, who are therefore more productive. In support of this interpretation, Jager et al. (2004) suggested that low feeding levels exacerbated the pgr effects of toxicants in *F. candida*, using the data of Crommentuijn et al. (1997) from laboratory exposure to cadmium and triphenyltin.

The effect of ivermectin on fecundity is likely to be a result of its deterrent effect on feeding, shown in Fig. 5. Such avoidance behavior has been reported for *F. candida* for other toxicants in laboratory exposure tests (Fountain and Hopkin 2001, Heupel 2002, Smit et al. 2004). In the field, Collembola are naturally exposed to toxins occurring in certain species of fungi containing melanin. *Folsomia candida* have been reported to avoid these fungi in laboratory food preference tests (Scheu and Simmerling 2004). Avoidance behavior may explain why, in the field, collembolan numbers were found to be significantly lower in ivermectin-treated cow pats (1150 ng/g dry weight) compared to the control pats (Suarez et al. 2003). Declines in food consumption at the highest ivermectin concentrations may have caused a reduction in fecundity because *F. candida* must feed in order to reproduce (Wiles and Krogh 1998).

The contour plot in Fig. 3 is reminiscent of Tilman's (1982) plots of pgr in relation to the amounts of two resources. Tilman considered the case of rectilinear pgr plots, rather like Fig. 3 except that the axis directions are reversed, because increasing resources are beneficial, not stressful as for the axes of Fig. 3. Tilman showed that rectilinear pgr plots result if two resources are independent and both are essential. Although this mechanism only applies to resources, not to toxins such as ivermectin, and so is not applicable to Fig. 3, it is interesting that compensation and essentiality provide alternative explanations of rectilinearity in pgr plots.

The spacing of the pgr contours in the vicinity of the pgr = 0 contour determines the form of population regulation, specifying the way that pgr changes with density. For the theoretical background to this approach see May et al. (1974) and Lande et al. (2002*a*, *b*). Narrow spacing in the density direction indicates that the population will bounce back quickly after perturbation; wide spacing points to a slow response. The "strength of density dependence" can be calculated as

$$\frac{\partial(\text{pgr})}{\partial(\log_e \text{density})_{|\text{carrying capacity}}}$$

(May et al. 1974, Lande et al. 2002*b*). Interpolating from Fig. 3, pgr declines from +0.1 to −0.1 as density increases from ~25 to ~58. Thus Δ(pgr) is −0.2 and Δ($\log_e$ density) is ~0.84, so the strength of density dependence is ~0.24/wk. The reciprocal of the strength of density dependence indicates the time needed to recover equilibrium after a perturbation, here approximately four weeks. This is longer than the generation time (about three weeks) and so the population "undercompensates" for environmental perturbations (see e.g., Begon et al. 1996). In the field, however, a faster response has been reported, with Collembola numbers in treated pats returning to control cow pat levels seven days after treatment with ivermectin (Suarez et al. 2003). This response may be a result of interacting abiotic factors or immigration.

Over time, fecundity declined at high chemical concentrations (Fig. 5). This could be a result of delayed toxicity of ivermectin, or the consequence of increased energy expenditure on detoxification mechanisms. Springtails have the ability to store toxicants in the epithelial cells of the midgut and these are shed at each molt. This is a very effective method of ridding the individual of the unwanted substance (Hopkin 1997), but may use resources, including energy, that are consequently not available for reproduction (Walker et al. 2001).

Conclusions

Standard regulatory protocols, such as International Organisation for Standardisation (ISO) Collembola reproduction tests do not replicate field situations where Collembola populations may be regulated by density dependence and exist at carrying capacity. If less than additive effects occur in the field, standard regulatory protocols performed with growing populations or low density populations may overestimate the effects of toxicants (Hooper et al. 2003). However, prior to attempting generalizations, more tests over a wide range of toxicants and life history types are required. Studying high-density populations is more realistic of field conditions especially for ivermectin where effects are localized in cow pats, and springtails are likely to reach high population densities due to abundance of food. Soils that contain high levels of organic matter may increase the growth of *F. candida* (Booth and Anderson 1979, Chen et al. 1995, Kaneda and Kaneko 2002, 2004) and reduce toxic effects of toxicants (Martikainen 1996).

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