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# *FOLSOMIA CANDIDA* (COLLEMBOLA): A "Standard" Soil Arthropod*

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**Key Words** springtail, ecotoxicology, chemical toxicity, Isotomidae, ISO test

■ **Abstract** *Folsomia candida* Willem 1902, a member of the order Collembola (colloquially called springtails), is a common and widespread arthropod that occurs in soils throughout the world. The species is parthenogenetic and is easy to maintain in the laboratory on a diet of granulated dry yeast. *F. candida* has been used as a "standard" test organism for more than 40 years for estimating the effects of pesticides and environmental pollutants on nontarget soil arthropods. However, it has also been employed as a model for the investigation of numerous other phenomena such as cold tolerance, quality as a prey item, and effects of microarthropod grazing on pathogenic fungi and mycorrhizae of plant roots. In this comprehensive review, aspects of the life history, ecology, and ecotoxicology of *F. candida* are covered. We focus on the recent literature, especially studies that have examined the effects of soil pollutants on reproduction in *F. candida* using the protocol published by the International Standards Organization in 1999.

## INTRODUCTION

Collembolans are among the most abundant arthropods on Earth with a long evolutionary history (31). Most species consume fungi in soil and leaf litter, but they have radiated into many niches, from the littoral zone to mountaintops, and are particularly abundant in epiphytes of tropical rain forests (54). Collembolans

*Abbreviations: a.i., active ingredient of a commercial pesticide; EC50, concentration of a substance that causes a 50% reduction in reproduction (effect concentration). In the ISO *F. candida* test, the EC50 is the estimated concentration that results in a 50% reduction of juveniles compared with the controls. LC50, lethal concentration of a substance that causes a 50% reduction in survival. In the ISO *F. candida* test, the LC50 is the estimated concentration that results in a 50% reduction in the number of adults still alive at the end of the experiment compared with the controls.

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certainly represent a monophyletic lineage that was an early branch off the line that led to the higher insects. However, there is no consensus whether this occurred before or after the divergence of the Crustacea (27, 83, 84).

Collembolans are an integral part of soil ecosystems and are vulnerable to the effects of soil contamination. The abundance and diversity of Collembola have been used widely to assess the environmental impact of a range of pollutants on soils. However, field effects are difficult to reproduce in the laboratory. Anthropogenic activities and the need for tighter controls on waste and chemical emissions have led to the search for biological test species. An organism's responses to chemicals in the laboratory can be used to assess stress and inform legislative processes (123–125).

For a number of years, interest has been shown in the unpigmented springtail *Folsomia candida* Willem 1902 (Figure 1; Figures 2 and 3, see color insert). The collembolan can be exposed to contaminants via the soil and/or food in a battery of tests that examine life-history parameters, bioaccumulation, and/or effects on behavior. Such tests assess the toxicity of a wide range of organic and inorganic pollutants and have been used as bioassays to monitor the success of remediation of contaminated soils (24, 28, 32–34, 39, 41, 62, 66, 96, 121) (Table 1). The International Standards Organization (ISO) has recently published a protocol for the use of *F. candida* as an ecotoxicological test species that employs effects on reproduction as an endpoint (58).

However, *F. candida* has been used extensively as a model arthropod in many other nonecotoxicological studies, including work on the evolution of *Hox* genes (40). In this review, we provide an overview of all aspects of the biology of

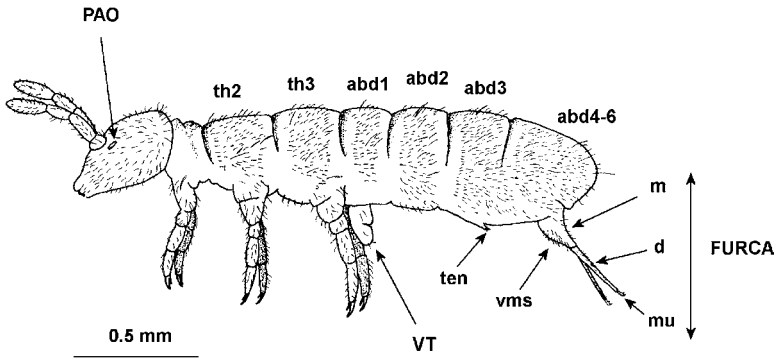


**Figure 1** Adult female *F. candida*. In the living animal the furca is held in place under the body by the tenaculum (ten). The first thoracic segment is reduced dorsally compared with the second (th2) and third (th3). The last three abdominal segments (abd4–6) are fused together. d, dens; m, manubrium; mu, mucro; PAO, post-antennal organ; vms, ventral manubrial setae; VT, ventral tube. (Modified after Figure 1 in Reference 112 with permission of the authors).

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**TABLE 1** Effects of metals and organic chemicals on reproduction in *F. candida* in order of their toxicity (EC50 mg $kg^{-1}$ dry mass of test soil at 20°C) using the ISO protocol (58), or broadly similar experimental conditions

| Metals | | | Organics | | |
|---|---|---|---|---|---|
| **Chemical** | **EC50** | **Reference(s)** | **Chemical** | **EC50** | **Reference(s)** |
| Cu oxychloride | 1.35 | (57) | Euparen | 0.03 | (57) |
| Hg | 3.26 | (73) | Nimbecidine | 0.09 | (57) |
| As (III) | 13.1 | (78) | Thiodan | 0.14 | (57) |
| Cd | 51–780 | (19, 20, 23, 72, 94, 95, 119) | Confidor | 0.15 | (57) |
| | | (120, 122) | E 605 Forte (a.i. parathion) | 0.10–0.18 | (58) |
| Zn | 50–2088 | (74, 75, 94, 95, 103, 104, 105) | Lindane (OC) | 0.189 | (71) |
| | | (102, 106, 107, 119, 128) | Dimethoate | 0.4–6.3 | (68, 81, 82) |
| Cu | 250–1480 | (13, 94, 95) | Nonylphenol | 2.9 | (43) |
| Ni | 476 | (77) | HHCB | 60 | (6) |
| Pb | 580–3160 | (9, 94, 95) | Pentachlorophenol | 87 | (23) |
| Cr (III) | 604 | (76) | LAS | 91 | (59) |
| Mn | 1663 | (70) | Phenol | 93.9 | (43) |
| | | | AHTN | 100 | (6) |
| | | | Betanal Plus | 36–200 | (57, 58) |

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*F. candida*. All work that we could find published since 1995 that refers to *F. candida* is cited. Earlier references are included in the reviews by Hopkin (54) and Wiles & Krogh (132).

## BIOLOGY

### Taxonomy and Distribution

The genus *Folsomia* includes species in the family Isotomidae that have a well-developed furca (springing organ), no anal spines, and an abdomen with the posterior three segments fused (Figure 1 and Figure 2). The original description of *F. candida* by Willem in 1902 (133) was based on a single specimen floating on a puddle in a cave at Rochefort in Belgium. The species is 1.5 to 3.0 mm in length at maturity, is white or faintly yellowish in color, and does not bear ocelli. There is a post-antennal organ behind the base of each antenna that probably detects airborne chemicals (54). Like all other collembolans, *F. candida* has a pair of thin-walled, closely apposed, eversible vesicles on the ventral side of the first abdominal segment. This structure is commonly known as the ventral tube, or collophore, and is involved in fluid exchange with the external environment. The ventral tube is an important exposure route for chemicals dissolved in soil pore water (79). The most distinguishing feature that separates *F. candida* from other members of the genus is the presence of numerous (at least 16) stout setae on the ventral side of the manubrium of the furca [*F. candida* is described in detail in *Synopsis on the Palaearctic Isotomidae* by Potapov (88)].

*F. candida* is considered a tramp species (54). Because it has been carried all over the world in plant pots and soil (see map at http://www.collembola.org/), its original biogeographical locations are difficult to ascertain (43). Many records are from caves and mines (112). Elsewhere the species inhabits agricultural systems, soils with a high level of organic matter, forests, and the edges of streams. *F. candida* is occasionally the dominant collembolan. In a recent study of collembolan diversity conducted in woodlands in Scotland, it was the most abundant species (67).

### Life History and Development

This description of the life history of *F. candida* is based on published work (54, 132) as well as observations on our laboratory cultures that have been maintained in Reading, United Kingdom, since 1994.

Populations of *F. candida* consist exclusively of parthenogenetic females. At 20°C (ISO standard test temperature) they take between 21 and 24 days to reach the sixth, or adult, instar when they are sexually mature. About 30 to 50 eggs are laid in each batch, which take 7 to 10 days to hatch. The eggs are white, spherical, and 80 to 110 $\mu$m in diameter (Figure 3). Eggs maintained above 28°C fail to hatch. The optimal temperature for hatching success is 21°C. At lower temperatures, the time span for each developmental stage is extended. For example, the average

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lifespan of a female at 15°C is 240 days, whereas at 24°C it is only 111 days. At 15, 21, and 27°C, the average number of eggs laid by a female during her lifetime is around 1100, 900, and only 100 (54, 132), respectively. Eggs are often laid in communal heaps, in which females add to previously laid batches. Crowding ($>1$ animal $cm^{-2}$) reduces the number of eggs laid, with some individuals developing malformed genital plates. The causes of this reduced fecundity may be stress from jostling, pheromones, or contamination of the substrate by waste products. There may also be increased cannibalism of eggs. Although *F. candida* is blind, more eggs are produced in constant darkness than in a light:dark cycle, which suggests the presence of internal photoreceptors.

An adult female may go through 45 molts in her lifetime with short reproductive instars (duration 1.5 days) alternating with longer nonreproductive instars (duration 8.5 days) (5). The lining of the midgut is also shed and voided in the feces during molting. This provides an important route of excretion of waste products and pollutants stored in the midgut cells as part of a storage detoxification system. Collembolans probably follow this strategy due to their lack of Malpighian tubules (52).

## Physiology and Behavior

Soil is often considered a stable environment. However, the surface layers are subject to wide fluctuations in temperature and moisture. Levels of oxygen and carbon dioxide may be variable in the pockets of gas within which *F. candida* survives periods of flooding.

All life stages of *F. candida* are well adapted to dry soil conditions (47). The species possesses physiological adaptations to desiccation and absorbs water vapor and remains active below 98.9% relative humidity (RH) (the permanent wilting point of plants). It can actively increase osmotic pressure of its body fluids, halt water efflux by the synthesis of myoinositol (a polyol undetectable in control animals at 100% RH), and reestablish hyperosmoticity within 48 h (7). *F. candida* has improved survival under drought stress, if it has been exposed previously to desiccation, owing to the induction of sugars and polyols. Below 95.5% RH, it switches from a hyperosmotic to an anhydrobiotic strategy with an increase in trehalose levels and a decrease in myoinositol (101). This mechanism of drought tolerance has an overlapping adaptation with cold tolerance at the cellular level by increasing the molar percentage of cryoprotective membrane fatty acids with a mid-chain double bond and stimulating the synthesis of the heat shock protein (Hsp) 70 (8, 51). Hsp70 may also be induced by exposure to pesticides (109).

Oxygen uptake is via the cuticle (*F. candida* does not possess tracheae). During hypoxia, individuals show increased heart contraction frequency, which helps maintain partial pressure differences between the surrounding external medium, blood, and tissues (86). Because collembolans do not possess respiratory pigments, the oxygen capacity of the extracellular fluids is low. However, some individuals can survive for up to 18 h in completely anaerobic conditions.

In soil, levels of carbon dioxide in pockets of trapped gas can be high (137). *F. candida* has evolved to survive in such conditions for considerable periods

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and is capable of becoming the dominant species in communities of Collembola subjected to elevated carbon dioxide (61). The species can survive up to 25% carbon dioxide for one hour or 10% carbon dioxide for six weeks (137).

## Interactions with Other Organisms

*Wolbachia* are obligatory, cytoplasmically inherited $\alpha$-Proteobacteria that infect the reproductive tissues of many arthropods (117). Those infecting collembolans form a monophyletic group and are found in the ovaries and brain. In *F. candida*, *Wolbachia* may be the cause of parthenogenesis (25).

Some researchers have attempted to use *F. candida* as a source of food for cultures of carnivorous invertebrates. However, recent work on predation by lycosid spiders has shown that *F. candida* contains toxins that may be poisonous to potential predators (60). The spiderlings of *Pardosa lugubris* fail to develop when fed a diet consisting exclusively of *F. candida* (85).

# FOOD AND FEEDING

## Digestive System

The digestive system of *F. candida* is essentially cylindrical with a cuticle-lined foregut and hindgut, and a midgut derived from endodermal cells that produce enzymes and absorb products of digestion. There are no gut diverticula. A peritrophic membrane, which is produced at the junction of the foregut and midgut, surrounds the food on its passage through the hindgut, where water is absorbed from the feces before they are voided.

The gut passage time of *F. candida* at 20°C is approximately 35 min (113). Waste products stored in the midgut cells (including pollutants) are voided into the lumen when the springtail molts and are lost in the feces. This excretory process is more effective if the animal is feeding regularly. For example, well-fed *F. candida* lost assimilated rubidium in 46 days, whereas starved animals needed 103 days to excrete the element completely (29).

The digestive tract of *F. candida* is host to a wide range of microorganisms whose numbers vary over the molt cycle. Heterotrophic aerobic gut bacteria increased from only $4.9 \times 10^2$ colony-forming units (CFUs) just after molting to $2.3 \times 10^6$ colony-forming units just before the next molt (113). Eleven taxonomically different bacteria and one filamentous fungus (*Acremonium charticola*) have been isolated from the digestive tract. A new technique developed with *F. candida* detects internal bacteria by means of small-subunit rRNA-targeted fluorescence in situ hybridization microscopy (114). This technique is faster and less damaging than more traditional techniques that use paraffin-embedded sections.

*F. candida* consumes and inactivates entomopathogenic fungi applied as biological pesticides without suffering mortality, reproductive disturbance, or any other harmful effects (11, 100). The species is unaffected by *Bacillus* toxins, including those produced in transgenic cotton (3, 136). The gut of *F. candida* is also a site of

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transfer of conjugative genes between microbes (48). The digestive tracts of soil arthropods may thus be important "hot spots" for gene transfer.

## Food Quality and Selective Grazing

Most collembolans feed on fungal hyphae. *F. candida* is no exception and exhibits strong preferences for particular species (see Reference 54 for a review of the earlier literature). In laboratory microcosms, *F. candida* prefers fungi growing on the surfaces of leaf litter rather than on soil particles (10). There is good evidence that *F. candida* is an important stimulant of decomposition. For example, the presence of *F. candida* in laboratory microcosms doubled the concentration of nitrates in leachates from decomposing grassland plant litter compared with controls with no collembolans (17). In the laboratory, *F. candida* consumes nematodes but it is not clear whether they are important components of the diet in the field (56). *F. candida* has also been used to test the efficacy of the bait lamina technique. This investigates the feeding activity of soil organisms on buried plastic strips containing food (44).

The fungus on which *F. candida* feeds influences its growth and fecundity. Laboratory experiments with *F. candida* held in different microcosms, with only one species of fungus available in each, have shown that some taxa of fungi are more nutritious than others. However, it is difficult to ascertain the reasons for these differences. They may be related to protein content (although not all studies have supported this relationship; 30) or inhibition of growth by chemicals that act as feeding deterrents (54).

## Grazing on Root Symbionts

Some studies have shown beneficial effects on plant growth of intermediate levels of collembolan grazing on root mycorrhizae. The level of infection of the symbiotic fungus is stimulated compared with plants grown in the absence of *F. candida*, or in the company of high population densities of collembolans (80). However, the effects are species specific and are not well understood (65).

There has been criticism of microcosm experiments on the effects of grazing by *F. candida* on root symbionts where no food source other than mycorrhizal fungi was present. Given the choice, *F. candida* preferred to consume the saprophytic fungus *Alternaria alternata* rather than arbuscular mycorrhizal fungi (AMF). Indeed, the springtails did not produce eggs when fed exclusively three species of AMF (64). It was concluded that previous "clean" studies had overestimated the effects of *F. candida* on AMF compared with conditions in the wild. Nevertheless, the grazing activity of *F. candida* was clearly beneficial to growth of ribwort plantain (*Plantago lanceolata*) by facilitating the transport of spores of essential AMF to neighboring plants (65).

## Control of Plant Pathogens

Collembolans have been implicated in the control of the bacterial fire blight pathogen *Erwinia amylovora*, which is highly destructive to fruit trees. The

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pathogen is digested in the gut of *F. candida*, contributing to a decrease of bacterial populations in the soil (46).

*Sclerotinia sclerotiorum* (white mold) is a fungus that attacks many plant species, including soybeans. It produces black fleshy structures called sclerotia that allow the fungus to survive from one cropping season to the next. The role of *F. candida* in transferring the mycoparasite *Coniothyrium minitans* between the sclerotia, and its use as a biocontrol agent, has been investigated. *F. candida* attained a larger size when fed *C. minitans* than when fed yeast and another mycoparasite. The collembolan also had the ability to inoculate uninfected sclerotia if transferred from infected treatments, since 60% of the conidia survive gut passage in a viable form in the feces (135). *F. candida* may transmit the conidia for a distance of at least 55 mm in soil (134).

A recent laboratory study on the suitability of fungi as food for *F. candida* has highlighted the importance of considering not only the species of fungus involved, but also its life stage (93). Four species of pathogenic fungi that cause rot disease of winter cereals were examined. The mycelia of three of the species were an adequate source of nutrition for reproduction in *F. candida*. However, the mycelia of *Bipolaris sorokiniana* were repellent and collembolans that fed on them died. Intriguingly though, the conidia of *B. sorokiniana* were eaten by *F. candida* and provided a sufficient diet for successful reproduction.

*F. candida* has proved to be an excellent model for demonstrating the wide range of responses that may result from collembolans grazing on mycorrhizae and other soil and leaf litter fungi. However, almost any outcome can occur. There can be stimulation or inhibition, the extent of which is influenced by the density of collembolans in the soil (and indeed the species of springtails present). One major challenge for soil biologists is to extrapolate the results of laboratory experiments on model species such as *F. candida* to the vastly more complicated situation in the field.

## ECOTOXICOLOGY

### Introduction

Ecotoxicology studies the effects of chemicals on organisms (129). In an ideal world all chemicals would be tested on all animals before being released into the environment. However, this is an impossible task. A compromise has been reached whereby representative species are used as screening tools with the aim of highlighting substances that are particularly toxic. For soils, earthworms (*Eisenia* sp.), enchytraeids (*Enchytraeus* sp., *Cognettia* sp.), and collembolans have been the most widely used groups because of their ease of culture in the laboratory and relatively short generation times at room temperature (1, 91). Several species of collembolans have been employed over the years, including the sexually reproducing *Folsomia fimetaria* (4, 5). However, most researchers have used *F. candida*, leading to the publication in 1999 of a recommended protocol by the International Standards Organization (ISO) (58).

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## The ISO Test

ESTABLISHING AND MAINTAINING CULTURES A laboratory conducting toxicity tests with *F. candida* needs a continuous supply of animals from productive cultures. These are simple to maintain. Rectangular clear plastic containers (about 400 ml in volume) with tightly fitting lids are filled with a mixture of plaster of Paris and activated charcoal (or powdered graphite) in a ratio of 9:1 by weight, mixed with an approximately equal volume of distilled water (some formulations of plaster of Paris contain zinc as a bactericide, which may interfere with results). The charcoal absorbs waste gases and excretion products and facilitates observation of the white springtails against the dark background. The surface of the plaster of Paris should be scored with a knife before it sets to provide furrows for springtail oviposition.

Most cultures die out through desiccation. A small volume of free water should always be present so that the substrate is permanently saturated. Tilting the containers slightly with small supports ensures that the water collects at the lower end, leaving a drier area at the other end where eggs can be laid without becoming waterlogged. A few milligrams of granulated dry yeast (widely available from food stores) are placed on the surface of the plaster of Paris at the dry end. A few (typically 10) adult *F. candida* are introduced to the containers. Most laboratories with cultures of *F. candida* will donate these. Although there are small differences in the responses of clones from different sources, these are not sufficient to be considered a significant problem (14, 21, 98, 110). This is in marked contrast to the *Daphnia magna* test, in which differences in responses to chemicals between clones may be more than two orders of magnitude (129). The containers are maintained at 20°C in a constant temperature room in continuous light or under a 16:8 h light:dark cycle. The lids should be removed every two to three days to aerate the cultures and replenish water and food if necessary.

Transfer of females to fresh containers usually induces oviposition, and eggs are soon visible under a microscope as small clusters of pale yellowish spheres (Figure 3). After 7 to 10 days the eggs hatch and after three weeks these juveniles are mature and begin to lay eggs of their own. Tipping out most springtails from time to time to reduce the population density prevents overcrowding. Every few months, it is advisable to set up fresh cultures to avoid the reduced levels of oviposition that sometimes occur in containers that have been maintained for a long time and become tired. Moving a culture into a refrigerator at 5°C for two days and then moving it back to room temperature often stimulates *F. candida* to start laying eggs again.

PERFORMING THE ISO REPRODUCTION TEST A variety of routes of exposure of *F. candida* to chemicals have been studied. These include food, gas, water, contaminated leaf surfaces over which the collembolans were forced to walk, and topical application of substances onto individual springtails (16, 36, 55, 57, 111, 115). However, the ISO test deals exclusively with contact with contaminated pore water in soil, as this appears to be the most toxic route of exposure (92). Results

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from tests with contaminated food may underestimate toxicity, as springtails can "taste" the chemicals in the diet and avoid it (35).

The test substrate is an artificial soil comprising (by air-dried mass) (*a*) 10% *Sphagnum* peat, finely ground with no visible plant remains; (*b*) 20% kaolinite clay containing not less than 30% kaolinite; and (*c*) 70% industrial quartz fine sand with more than 50% of particles from 0.05 mm to 0.2 mm in diameter. Pulverized calcium carbonate is added (usually 0.5% to 1%) to bring the pH (as measured in 1 mol liter$^{-1}$ KCl solution) to 6.0 $\pm$ 0.5 (the exact amount to be added is determined by measuring the pH of subsamples of the hydrated artificial soil).

*F. candida* shows a mild preference to settle on soils of pH 5.6, at which females achieve their highest level of reproduction compared with more acidic or alkaline conditions (43, 127). The components are thoroughly mixed, and distilled water is added until the soil has a crumbly structure to enable springtails to penetrate substrate cavities. The amount is normally around 35 ml per 100 g of dry soil, which is equivalent to 40% to 60% of the total water-holding capacity, but this is not absolutely critical (122).

The chemical to be tested is dissolved in the water at the range of concentrations that will give the appropriate levels in the hydrated soil. Substances that are insoluble in water are dissolved in organic solvents and mixed with soil. Water is added after the solvent has evaporated. An even distribution of the contaminant within the soil is not crucially important (68). The ISO protocol does not define the length of any aging process for the substrate, i.e., the time interval between making up the contaminated soils and adding the springtails. Aging of soils has a considerable influence on the toxicity of added chemicals (74, 104, 106), so its omission is important. A time of 48 h between making up the soil and introducing the springtails would seem to be appropriate.

The glass test vessels should be 100 ml in volume (about 5 cm in diameter) with tightly fitting lids. Each container is filled with 30 g wet mass of soil. The appropriate concentrations of test substance to use in the final assay test can be assessed by conducting a preliminary range-finding exercise (57). The number of concentrations used in the final test will depend on the results of the preliminary test. However, a minimum of a control plus a geometric series of four ascending concentrations with five replicates of each (i.e., 20 test containers in total) is recommended. The more replicates employed, the more statistically valid the test (116), but the final number will be constrained by the resources available.

The test is conducted with 10- to 12-day-old *F. candida*. These are obtained by initially placing numerous adult *F. candida* into fresh culture vessels. The females are allowed to oviposit for 48 h and are then removed. About one week later the eggs hatch and after an additional 10 days the juveniles are ready to be used in the test. Evidence suggests that the age of these animals has a considerable influence on the outcome of the test (22). Ten springtails are added to each replicate, about 2 mg of dry yeast is placed on the soil surface, and the lids are closed. Twice a week, the lids are removed for aeration. After two weeks, fresh yeast is added.

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The test is run at 20°C under a light:dark cycle of between 12:12 and 16:8 h (intensity 400–800 lux) for 28 days, allowing sufficient time for the springtails to lay up to two batches of eggs. The soil from each container is mixed with 500 ml of water in larger vessels and the live adults and juveniles float to the surface. Alternatively, the springtails can be heat-extracted from the soil using Tullgren funnels. In the past, most researchers have taken photographs or have manually removed the springtails for counting. Currently, most researchers employ computer-aided image analysis, as this is both faster and more reliable (69).

For the test to be valid for legislative purposes, the mortality of the adults in the controls should not exceed 20%, there should be at least 100 juveniles in each control vessel, and the coefficient of variation of reproduction in the control should not exceed 30%. The EC50 concentration of the test substance is that which reduces the reproductive rate at the end of the test by 50% compared with the control (Figure 4). The toxicities of the agricultural chemicals Betanal Plus (a.i. 160 g liter$^{-1}$ phenmedipham) and E 605 Forte (a.i. 507.5 g liter$^{-1}$ parathion) have been determined in a ring test (Table 1). These compounds are recommended as reference substances to be tested on *F. candida* at least once a year for quality assurance (58).

RESULTS OF THE ISO TEST The ISO test has been used to assess the toxicity of a wide range of substances to *F. candida*. In general, the springtail is much more sensitive to the effects of organic chemicals than to metals (Table 1). The large ranges for EC50s for some chemicals (particularly cadmium, copper, lead, and zinc) are due to differences in the forms of the substances used, the types of soil employed in the tests (some of the studies used natural soils rather than the ISO medium), and other factors. These include variations in pH, temperature,

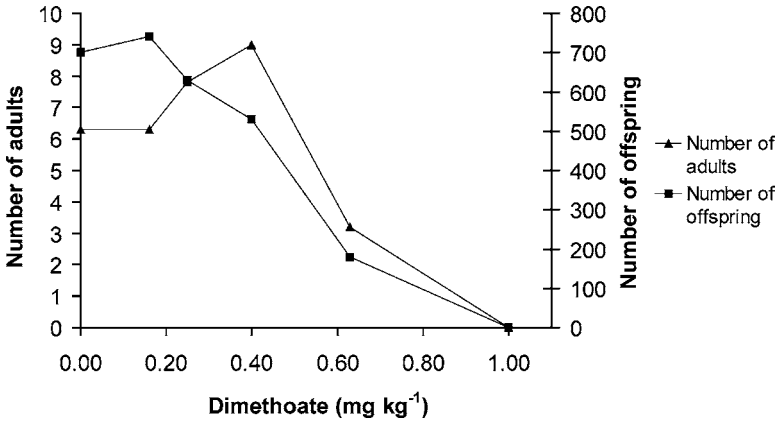


**Figure 4** Toxicity of dimethoate to *F. candida*. The EC50 for juvenile production is approximately 0.5 mg kg$^{-1}$ (modified after Figure 1 in Reference 68 with permission of the author).

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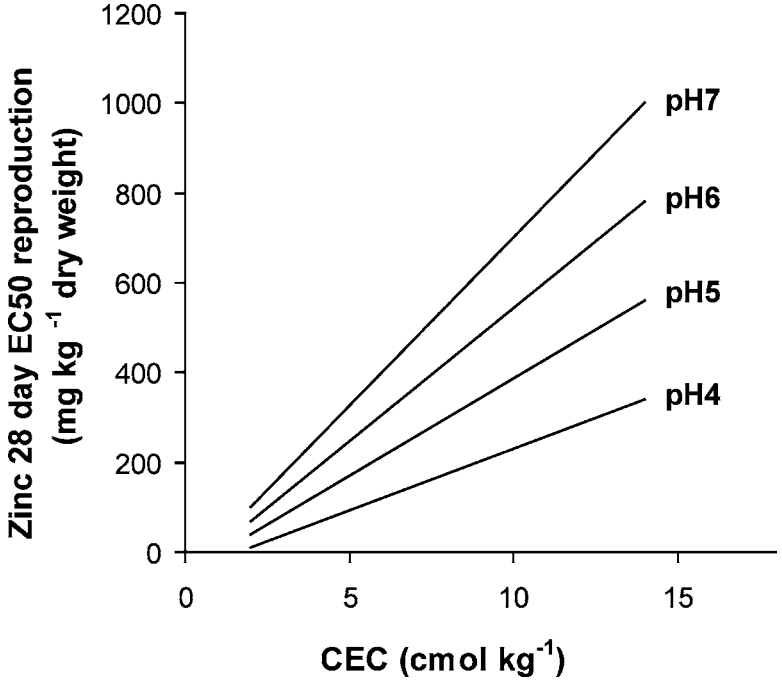


**Figure 5** Model of the relationships between pH and cation exchange capacity (CEC) of soils, and the effects of zinc on reproductive output in *F. candida* (modified after Figure 1 in Reference 74 with permission of the authors).

moisture, length of aging of soils before introduction of springtails, and organic matter (OM) content (74, 94, 95, 103, 104, 106, 122). In general, small variations of 10% of the ISO values for temperature and moisture do not have a disproportionate influence on EC50s. However, the outcomes of the test are much more sensitive to changes in pH, cation exchange capacity, and OM content (Figure 5). In general, for metals, the lower the pH and OM content of the soil, the more negative the effect on reproduction of *F. candida* at a given concentration. The counterion is also influential; lead nitrate is more toxic to *F. candida* than lead chloride (97). However, this rule does not follow for all pollutants. Indeed, some organic chemicals such as dimethoate and parathion become more toxic as soil OM increases (81, 92).

Bioaccumulation of chemicals can be determined by analyzing concentrations in the collembolans at the end of the experiment. This may be important in predicting food chain transfer to their predators. However, *F. candida* is a small animal and it is a considerable technical challenge to determine the levels of substances in such tiny samples. Nevertheless, graphite furnace atomic absorption spectrometry is sensitive enough to measure accurately concentrations of metals in individual springtails (36). Studies have shown that while nonessential metals such as cadmium and lead are accumulated roughly in proportion to their values in soil pore water, the essential elements copper and zinc are regulated (128). Indeed,

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*F. candida* maintains stable internal levels of copper at food concentrations that have detrimental effects on their growth and reproduction (13). There is little evidence of antagonism between cadmium and zinc when they are both present in the soil, which suggests that the routes of assimilation do not strongly compete (119).

## Field Relevance of the ISO Test

The main purpose of carrying out ecotoxicological tests on *F. candida* is to provide data for risk assessment. The risk quotient (RQ, the measure of environmental risk) is determined from the predicted environmental concentration (PEC) of a chemical and the predicted no-effect concentration (PNEC) of the same chemical, in this case for *F. candida* (129).

RQ is calculated using the following formula:

$$RiskQuotient(RQ) = \frac{PNEC}{PEC}$$

If $RQ > 1$, the risk is acceptable. If $RQ < 1$, the risk is unacceptable. The PEC for soils is determined after consideration of field application rates (in the case of pesticides), deposition rates (in the case of aerial pollutants), or existing levels of contamination (for example, mine spoil). The PNEC is derived from standard tests and may include a safety factor of 10, 100, or even 1000, but it should be higher the less that is known about the chemical. For example, tebufenozide (a new molt-inducing insecticide that mimics the action of ecdysone) had no effect on *F. candida* at 1000 times the PEC, which suggests that under normal conditions it should not pose a hazard to soil invertebrates (2). The toxicities of chemicals to *F. candida* are, in almost all cases, considerably higher in the ISO soil than in natural soils (12, 13, 63, 87, 128). Thus, the ISO test includes a built-in safety factor that should be taken into account when setting PNECs. However, large safety factors cannot be applied uncritically to essential elements such as copper. The predicted PNEC may be below the limit of essentiality (53)!

Tests on *F. candida* provide direct information only about the effects of chemicals on *F. candida*. How representative is this species of all Collembola? Most studies show that *F. candida* is among the most sensitive springtails to the majority of chemicals (15). *F. candida* is ten times more sensitive to chlorpyrifos than *Xenylla grisea* (99), and it is more sensitive to this organophosphate than *Isotomurus palustris* and *Isotoma viridis* (131). Nevertheless, there are exceptions. *Orchesella cincta* is around four times more sensitive to cadmium in food than *F. candida* (21).

Regarding other soil invertebrates, *F. candida* again is among the most sensitive taxon. For example, the LC50 for euparen (a.i. 500 g $kg^{-1}$ tolylfluanid, used as a protective fungicide in vineyards and orchards) for the standard test earthworm *Eisenia foetida* is $>1000$ mg $kg^{-1}$, whereas the LC50 for *F. candida* is only 0.072 mg $kg^{-1}$ (57). Euparen is even more toxic for reproduction (Table 1).

The ISO test with *F. candida* provides useful data to inform the development of PNECs. However, it is important to emphasize that PNECs should be proposed for individual species and chemicals on a case-by-case basis following informed

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debate. This should incorporate results of all laboratory tests, likely field exposure (including concentrations in soil pore water), biology of the organisms involved, and their interactions with other species (90, 92, 118, 126, 130). It is dangerous to extrapolate. It cannot be assumed that if reproduction is the most sensitive parameter in one species that it will also be in another (18).

## CONCLUSIONS

*F. candida* is a widespread and common animal. In ecotoxicology, it has been possible to relate soil pollution levels to the point along a pollution gradient where the species dies out (37, 38, 42). Indeed, *F. candida* is increasingly being used as a bioassay of soil remediation methods. For example, addition of metal-immobilizing agents to zinc-contaminated soil greatly reduces toxicity of this metal to *F. candida* (79).

*F. candida* has proved to be useful in highlighting how stresses not experienced in laboratory standard tests, such as dehydration, may increase sensitivities to chemicals (26, 49, 50). Future testing methods will involve increased use of more sophisticated endpoints that are affected by concentrations of contaminants lower than the EC50 (45, 108, 115). These tests are likely to include locomotory behavior (108), avoidance of contaminated food (35, 36), and effects of chemicals on population growth rates (19).

Although there has been some criticism toward the field relevance of the ISO test with *F. candida*, this species still has important roles to play in risk assessment of industrial chemicals (22, 94, 95) and genetically modified crops (89). *F. candida* will continue to be employed in the development of new environmental quality standards such as *Species Sensitivity Indices* (123, 125). The ease with which *F. candida* can be reared in the laboratory ensures that the species will continue to be exploited as a standard arthropod for many years to come.

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**Figure 2** Adult female *F. candida* with juveniles; the largest specimen is 2.0 mm in length (photo by S. Hopkin).

**Figure 3** Adult female *F. candida* of 1.5 mm in length next to a batch of eggs (photo by S. Hopkin).