

# Biology and Control of Root Weevils on Berry and Nursery Crops in Oregon

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## Abstract

The root weevils, *Otiorhynchus sulcatus* and *Otiorhynchus ovatus*, the black vine and strawberry root weevil, respectively, are perennial, ubiquitous pests of berry and nursery crops throughout the world. Recently, we developed a mass rearing system for these pests and now are able to conduct controlled experiments to study their biology and to develop chemical and biological management systems. Here we highlight our findings from these studies. We tested the hypothesis that feeding on multiple hosts may promote reproductive success. We used three hosts: strawberry, rhododendron and birds nest spruce (*Picea abies* 'Nidiformis') in a permuted array where adults were placed on a host for 15, 30, 45 or 60 days and then moved to another host for the remainder of their lives, or up to 300 days. Strawberry was the best sole host. Birds nest spruce alone, and in combination with other hosts, adversely affected reproductive processes. Some combinations of strawberry and rhododendron appeared to have a 'synergistic' effect when considering egg production. We devised a soil bioassay for screening pesticides or biological agents. In our first studies with the system we tested two formulations of bifenthrin. Granular bifenthrin prevented only the establishment of larvae, whereas the liquid formulation was efficacious on later instars. We have also performed field studies to compare the efficacy of bifenthrin and the entomopathogenic fungus *Metarhizium anisopliae* in which we observed no significant difference between chemical and fungal treatments. Laboratory bioassays were also performed to assess the virulence of *M. anisopliae* against field collected *O. ovatus*. The fungus was highly virulent even at the low dose of  $1 \times 10^4$  spores per g dry soil.

## INTRODUCTION

The root weevils, *Otiorhynchus sulcatus* (Fabricius) and *Otiorhynchus ovatus* (Linnaeus), the black vine and strawberry root weevil, respectively, are flightless and have one generation per year and apomictic parthenogenesis (Downes, 1922; Smith, 1932; Suomalainen et al., 1987). Eggs are laid in the early summer to fall. Overwintering occurs during the larval stage and pupation and transformation to adult occurs in the late spring to early summer. Both species appear to be omnivorous phytophagous insects in that a number of plant hosts have been listed for each species. However, a number of studies have found that evidence of damage, particularly by adults, does not necessarily denote that the plant is a viable larval host. The fecundity of *O. sulcatus* has even been shown to differ on cultivars of the same host (Cram, 1980; Nielsen and Dunlap, 1981; Cram and Daubeney, 1982; Shanks and Doss, 1986). In both the Eastern USA and in Europe, *O. sulcatus* appears to feed primarily on a number of angiosperms belonging to the plant orders Rosales, Primulales, Saxifragales and to a lesser extent the Ericales. In those studies, strawberry was not the premier host (Smith, 1932; Maier, 1981; Nielsen and Dunlap, 1981; Hanula, 1988; Van Tol et al., 2004). A recent study by Fisher (2006) concluded that in Western North America *O. sulcatus* appears to favor certain Rosales,

i.e., strawberry, blackberry and raspberry; Ericales, i.e., certain rhododendron species, blueberry and salal (*Gaultheria shallon*); and members of the gymnosperm families, Taxaceae and Pinaceae. In these studies we tested the hypothesis that feeding on multiple hosts may promote reproductive success.

There have been numerous studies on chemical control of *O. sulcatus* (Moore, 1990). However, due to the cryptic nature of the larvae, their supply has been limited; thus, efficacy in soil bioassays was not explored. We now mass-rear this insect (Fisher and Bruck, 2004). Consequently, we developed a soil bioassay for evaluating efficacy of biological and chemical control products directly on larvae of any chosen stage.

We have also been interested in finding efficacious biological agents for both weevil species. Nematodes are limited by low soil temperatures and have proven to vary in efficacy as they have a limited active period in the fall and late spring. However, the fungus *Metarhizium anisopliae* (Metchnikoff) Sorokin has been shown to persist well in potting media (Bruck, 2005). *M. anisopliae* is also effective against other beetles (Krueger and Roberts, 1997). A strain (F52) of this fungus has been registered for commercial sale in the United States. Here we tested the efficacy of this product against field-collected larvae of *O. ovatus* and in field trials compared the efficacy of *M. anisopliae* and bifenthrin against *O. sulcatus* in containerized plants that were confined *al fresco*.

## MATERIALS AND METHODS

### Host Plants and *Otiorynchus sulcatus*

We used three hosts, strawberry (*Fragaria × ananassa* (Duchesne) 'Totem'), rhododendron (*Rhododendron catawbiense × griffithianum* 'Cynthia' [RHS 58]), and birds nest spruce (*Picea abies* (L.) 'Nidiformis'). In a permutated array, teneral adults of *O. sulcatus* from our colony were placed on a host for either 15, 30, 45, or 60 days and then moved to another host until death or up to 300 days. Weevils were individually maintained in 100 x 25 mm vented Petri dishes containing a piece of moistened filter paper (Whatman #1) and a leaf or sprig of a selected host plant that was in a floral water-pic (Syndicate Sales Inc., Kokomo, IN). Adults were maintained in an incubator at 21±0.5°C with a photoperiod of 18:6 L:D (Nielson and Dunlap, 1981; Umble and Fisher, 2002) for the prescribed treatment period and then moved to a new host. Every 3 to 5 days, the dishes were examined for eggs, the plant material renewed, and the dishes changed to minimize mold and fecal deposits. Two parameters were recorded, longevity (in days) and eggs/♀. All data were submitted to GLM ANOVA (NCSS, 2004). After analysis, means were compared with the Dunnett's test ( $P \leq 0.05$ ) (NCSS, 2004) against the means for egg production and longevity when fed only one host.

### Soil Bioassay: Pesticides and *Otiorynchus sulcatus*

The commercially formulated insecticide Talstar® F (bifenthrin, 7.9% a.i., FMC Corporation, Philadelphia, PA) was used. This pesticide is the vine weevil control product of 'choice' by growers in our region. For this trial we used a soilless potting medium. The dilution series used was 0, 5, 10, 15, 20, and 30 mg L<sup>-1</sup> (formulated product). There were 5 replications of each treatment per trial with four trials performed in all. Each treatment consisted of a standard Petri dish (100 x 25 mm) with 20, ~ 3–4<sup>th</sup> instars placed on the treated potting media. A slice of carrot was added for nourishment. The experiment was then maintained at 21°C for 7 days and mortality recorded at 1, 3, 5, and 7 days. Linear regression analysis was used to analyze the data (NCSS, 2004).

An assay was also devised to test for insecticide efficacy against hatchlings. The test arena was a 5 cm<sup>2</sup> box containing fine sieved (120 mesh) field soil and Talstar® G (bifenthrin 0.2% a.i.) at 0, 25, and 50 mg L<sup>-1</sup>. After insecticide incorporation, the soil was moistened and 20 eggs that were near hatch were placed on top of the soil. Each day dishes were checked for egg hatch and larval mortality. The data were analyzed with regression analysis (NCSS, 2004). We present the data from 1, 4, and 8 days after

treatment.

### Soil Bioassay: Entomopathogenic Fungi and *Otiorhynchus ovatus*

*M. anisopliae* (strain F52, granule formulation, Earth BioSciences, Fairfield, CT) was used in laboratory soil bioassays against *O. ovatus*. Spores were washed from the granules in 0.1% Tween 80 solution. Spore viability was determined the day prior to performing the bioassay and the spore concentration adjusted to reflect viability (Goettel and Inglis, 1997). Spore viability was 90–95%.

Last instar *O. ovatus* were collected from an infested strawberry field and maintained in Petri dishes lined with moistened filter paper at 4°C for up to 2 days. Bioassays were performed using an autoclaved (1.1 kg/cm<sup>2</sup>, 121°C for 2 h, left overnight and autoclaved for an additional hour) field soil (Canderly Sandy Loam [66.3:22.5:11.2, Sand:Silt:Clay]). The bioassay included 0.1% Tween 80 solution as an untreated control. The treatments (one cup with 20 g of dried soil placed in a 30 ml plastic cup were inoculated to achieve either  $1.0 \times 10^7$ ,  $10^6$ ,  $10^5$  or  $10^4$  viable *M. anisopliae* spores g<sup>-1</sup> soil at 15% final moisture with ten last instars) were arranged in a randomized complete block design with four replicates (one cup per treatment). The cups for each replicate were placed inside a sealed 3.76 L zip lock plastic bag and maintained in complete darkness at 21°C for 14 days at which point the assay was evaluated for larval infection. Data were transformed to arcsine of percent larval infection to stabilize variance. Data from the bioassay were analyzed using GLM ANOVA with Tukey's multiple range tests used to separate means (SAS Institute, 1999).

### Container Studies

The efficacy of *M. anisopliae* and bifenthrin (Talstar® G) as media incorporations at planting for preventing *O. sulcatus* infestations were compared in outdoor trials. The granule formulations of both products were tested at rates recommended by the manufacturer. The experiment comprised four treatments: an untreated control, 227g per 0.765 m<sup>3</sup> *M. anisopliae* granules, 454 g per 0.765 m<sup>3</sup> *M. anisopliae* granules and 25 mg L<sup>-1</sup> of bifenthrin. Rooted cuttings of *P. abies*, 'Nidiformis', were then planted into #1 containers containing the various treatments and maintained inside 1.8 x 1.8 x 1.8 m mesh field cages. Twenty-five *O. sulcatus* eggs obtained from our colony (Fisher and Bruck, 2004) were applied to the surface of each pot on four occasions between May and July 2004. The plants were maintained outdoors in the field cages until they were evaluated for the number of live larvae in each pot the following spring. The experiment was arranged in a randomized complete block design with four replicates. Data were analyzed GLM ANOVA with Tukey's multiple range test used to separate means (SAS Institute, 1999).

## RESULTS AND DISCUSSION

### Host Plants and *Otiorhynchus sulcatus*

When the adult weevils were fed the hosts *F. x ananassa* and *R. catawbiense* for their reproductive life, they had the same longevity and produced nearly the same amount of eggs (Table 1). When fed the sole host *P. abies*, longevity was reduced and egg production was reduced by nearly 60%. The combination of feeding on *F. x ananassa* and then on *R. catawbiense* produced large quantities of eggs that were numerically higher than strawberry and significantly higher than the exclusive diet of *R. catawbiense* or *P. abies*. All combinations with *P. abies* either initially or later in reproductive life with *F. x ananassa* produced significantly fewer eggs and shortened life expectancy when compared to a diet exclusively of *F. x ananassa*; except for the short period on *P. abies* (30 days) and then the rest of the time on *F. x ananassa*. Combinations of *R. catawbiense* and *P. abies* produced varying results in egg production compared to an exclusive diet of *R. catawbiense*. Clearly *P. abies* was a poorer host for reproductive success than either *F. x ananassa* or *R. catawbiense*. However, it appears from this and previous work (Smith, 1932; Cram, 1965; Shanks, 1980; Hanula, 1988; Van Tol et al., 2004) that a cuisine of

multiple hosts such as weeds and plants that are in and near borders of production fields may contribute to either exacerbating or diminishing the pest status of *O. sulcatus*.

#### **Soil Bioassay: Pesticides and *Otiiorhynchus sulcatus***

Analysis of the bifenthrin flowable (Talstar® F) data found that there were significant differences in larval mortality among treatments for days after treatment and for increasing concentration (Fig. 1a). It should be noted that mortality did not approach 100% after 7 days exposure; even at 30 mg L<sup>-1</sup>. Since the 5 and 7 day observations paralleled each other (1 day,  $y = 0.41 + 0.17x$ ,  $R^2 = 0.95$ ; 5 day,  $y = 20.21 + 1.12x$ ,  $R^2 = 0.88$ ; 7 day,  $y = 45.51 + 0.99x$ ,  $R^2 = 0.78$ ) we suggest that increasing amounts of this formulation (even beyond the recommended rates) may control most *O. sulcatus* present. However, we are finding that some biological pesticides may be as effective (see below).

In the bioassay assessing the efficacy of bifenthrin in granular formulation (Talstar® G) against hatchlings, all treatments were significantly different from each other on each day [y intercept ± SE, 0 mg L<sup>-1</sup> = 0.03 (3.64), 25 = 46.28 (0.26), 50 ppm = 56.37 (1.8)] (Fig. 1b). However, it appears that it would take longer than 8 days to achieve 100% mortality. Fifty ppm paralleled the effect of 25 ppm but not at double the mg L<sup>-1</sup> effect (0 mg L<sup>-1</sup>,  $y = 0.03 + 1.58x$ ,  $R^2 = 0.83$ ; 25 mg L<sup>-1</sup>,  $y = 46.28 + 3.81x$ ,  $R^2 = 0.99$ ; 50 mg L<sup>-1</sup>,  $y = 56.38 + 3.89x$ ,  $R^2 = 0.75$ ). In fact, although significantly different, the effect was only half again that of 25 mg L<sup>-1</sup>. The data imply that using lesser quantities than the maximum recommended rate may provide some protection, but may not be as desirable as that afforded at 25 mg L<sup>-1</sup>. Certainly using more than 25 mg L<sup>-1</sup> would not be necessary to maintain efficacy.

#### **Soil Bioassay: Entomopathogenic Fungi and *Otiiorhynchus ovatus***

All treatments caused significantly higher levels of infection than the control ( $F = 14.34$ ; d.f. = 4, 15;  $P < 0.0001$ ), but there were no significant differences in larval infection among fungal treatments (Table 2). While there was a dose response to fungal inoculum level, the level of infection at  $1 \times 10^4$  was still > 70%, indicating that *O. ovatus* was highly susceptible to the commercial isolate of *M. anisopliae*. There was a single larva infected with *M. anisopliae* from the control treatment. This larva may have been exposed to *M. anisopliae* in the field, as entomopathogenic fungi are common in soils in the Pacific Northwest (Bruck, 2004).

#### **Container Studies**

The overall survival of *O. sulcatus* larvae in this experiment was poor. Even in the control treatment only 3.25 live larvae per container were recorded. However, most of the plants (> 75%) in the control treatment were severed at the soil surface and we believe that the majority of the larvae in the control died of starvation. This was not the case in the fungal- or bifenthrin-treated containers where none of the plants had succumbed to larval feeding. There was a significant treatment effect observed in this study ( $F = 4.84$ ; d.f. = 3, 12;  $P = 0.02$ ); the mean number of one larva per container was found in the high and low rates of fungal incorporation, but no larvae were present in the bifenthrin treatment. There was no significant difference between the fungal or bifenthrin treatments; however, all the treated containers had significantly lower larval populations than the control.

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#### **Literature Cited**

Bruck, D.J. 2004. Natural occurrence of entomopathogens in pacific northwest nursery

- soils and their virulence to the black vine weevil, *Otiorhynchus sulcatus* (F.) (Coleoptera: Curculionidae). *Environ. Entomol.* 33:1335–1343.
- Bruck, D.J. 2005. Ecology of *Metarhizium anisopliae* in soilless potting media and the rhizosphere: implications for pest management. *Biol. Control* 32:155–163.
- Cram, W.T. 1965. Fecundity of the root weevils *Brachyrhinus sulcatus* and *Sciopithes obscurus* on strawberry in the laboratory and outdoors. *Can. J. Plant Sci.* 45:169–176.
- Cram, W.T. 1980. Fecundity of the black vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae), fed foliage from some current cultivars and advance selections of strawberry in British Columbia. *J. Entomol. Soc. Brit. Col.* 77:25–26.
- Cram, W.T. and Daubeney, H.A. 1982. Responses of black vine weevil adults fed foliage from genotypes of strawberry, red raspberry and red raspberry–blackberry hybrids. *HortScience* 17:771–773.
- Downes, W. 1922. The strawberry root weevil with notes on other insects affecting strawberries. Canadian Department of Agriculture Pamphlet 5. New Series.
- Fisher, J.R. 2006. Fecundity, longevity and establishment of *Otiorhynchus sulcatus* (Fabricius) and *Otiorhynchus ovatus* (Linnaeus) (Coleoptera: Curculionidae) from the Pacific Northwest of the USA on selected host plants. *Agri. and Forest Entomol.* 8:281–287.
- Fisher, J.R. and Bruck, D.J. 2004. A technique for continuous mass rearing of the black vine weevil, *Otiorhynchus sulcatus*. *Entomol. Exp. Appl.* 113:71–75.
- Goettel, M.S. and Inglis, G.D. 1997. Fungi: Hyphomycetes. p.211–249. In: L.A. Lacey (ed.), *Manual of Techniques in Insect Pathology*. Academic Press, San Diego.
- Hanula, J.L. 1988. Oviposition preference and host recognition by the black vine weevil, *Otiorhynchus sulcatus* (Coleoptera; Curculionidae). *Environ. Entomol.* 17:694–698.
- Krueger, S.R. and Roberts, D.W. 1997. Soil treatment with entomopathogenic fungi for corn rootworm (*Diabrotica* spp.) larval control. *Biol. Control* 9:67–74.
- Maier, C.T. 1981. Reproductive success of the black vine weevil, *Otiorhynchus sulcatus* (F.), fed different foliar diets and evaluation of techniques for predicting fecundity. *Environ. Entomol.* 10:928–932.
- Moore, E.R. 1990. The potential of the entomogenous fungus *Metarhizium anisopliae* as a microbial control agent of the black vine weevil, *Otiorhynchus sulcatus*. University of Bath, UK. Ph.D. dissertation.
- NCSS. 2004. Number Cruncher Statistical System (Statistical Software). Kaysville, UT. USA.
- Nielsen, D.G. and Dunlap, M.J. 1981. Black vine weevil: reproductive potential on selected plants. *Ann. Entomol. Soc. Am.* 74:60–65.
- SAS Institute. 1999. The SAS Statistical System, version 8. SAS Institute, Cary, NC.
- Shanks, C.H.Jr. 1980. Strawberry and yew as hosts of adult black vine weevil and effects on oviposition and development of progeny. *Environ. Entomol.* 9:530–532.
- Shanks, C.H.Jr. and Doss, R.P. 1986. Black vine weevil (Coleoptera: Curculionidae) feeding and oviposition on leaves of weevil-resistant and -susceptible strawberry clones presented in various quantities. *Environ. Entomol.* 15:1074–1077.
- Smith, F.F. 1932. Biology and control of the black vine weevil. United States Department of Agriculture Technical Bulletin, 325.
- Suomalainen, E., Saura, A. and Lokki, J. 1987. Cytology and Evolution in Parthenogenesis. CRC Press, Boca Raton, FL.
- Umble, J.R. and Fisher, J.R. 2002. Influence of temperature and photoperiod on the preoviposition duration and oviposition of *Otiorhynchus ovatus* (L.) (Coleoptera: Curculionidae). *Ann. Entomol. Soc. Am.* 95:231–235.
- Van Tol, R.W.H.M., Van Dijk, N. and Sabelis, M.W. 2004. Host plant preference and performance of the vine weevil *Otiorhynchus sulcatus*. *Agric. For. Entomol.* 6:267–278.

## Tables

Table 1. Fecundity (eggs/♀) and longevity of *O. sulcatus* when fed a sole host versus being given alternate hosts at different times during their reproductive life (hosts are abbreviated but full names are given in the text).

Host 1	Host 2	Days on host 1	Egg/♀ ± 95% CI <sup>1</sup>	Dunnnett's test Control host <sup>2</sup>			Longevity (days) ± 95% CI	Ovipositing adults
				F. a.	R. c	P. a.		
<i>F. x a.</i>	-----	always	745.96 (101.56)		ns	* ^	228.4 (40.0)	26
<i>R. c.</i>	-----	“	611.96 (159.45)	ns		* ^	210.6 (45.5)	23
<i>P. a.</i>	-----	“	243.36 ( 62.51 )	*			191.4 (28.0)	22
<i>F. x a.</i>	<i>R. c.</i>	15	878.35 (125.57)	ns	* ^	* ^	251.5 (36.3)	26
“	“	30	882.38 (154.56)	ns	* ^	* ^	274.7 (34.3)	26
“	“	45	936.50 (162.60)	ns	* ^	* ^	194.6 (48.8)	20
“	“	60	720.20 (173.64)	ns	ns	* ^	213.6 (47.8)	20
“	<i>P. a.</i>	15	210.77 ( 49.14 )	*	*	ns	170.7 (30.7)	26
“	“	30	269.50 ( 56.35 )	*	*	ns	133.1 (39.3)	18
“	“	45	276.16 ( 54.35 )	*	*	ns	112.0 (25.8)	19
“	“	60	152.65 ( 45.77 )	*	*	ns	104.2 (24.8)	20
<i>R. c.</i>	<i>F. x a.</i>	15	566.72 (185.21)	ns	ns	* ^	187.1 (35.8)	25
“	“	30	157.47 ( 54.92 )	*	*	ns	118.9 (32.0)	19
“	“	45	372.61 ( 58.47 )	*	ns	ns	146.7 (33.6)	18
“	“	60	678.00 (181.40)	ns	ns	* ^	170.1 (42.6)	21
“	<i>P. a.</i>	15	153.63 ( 25.73 )	*	*	ns	115.2 (21.5)	22
“	“	30	163.15 ( 46.15 )	*	*	ns	113.8 (22.0)	20
“	“	45	170.70 ( 40.68 )	*	*	ns	135.3 (21.2)	23
“	“	60	146.42 ( 30.35 )	*	*	ns	129.2 (13.9)	26
<i>P. a.</i>	<i>F. x a.</i>	15	430.58 (124.51)	*	ns	ns	145.6 (38.8)	19
“	“	30	509.05 (156.35)	ns	ns	* ^	159.4 (42.0)	19
“	“	45	388.60 (158.09)	*	ns	ns	144.6 (38.1)	20
“	“	60	493.55 (153.20)	*	ns	ns	170.6 (38.1)	20
“	<i>R. c.</i>	15	886.40 (129.73)	ns	ns	* ^	232.6 (38.4)	20
“	“	30	777.80 (105.62)	ns	ns	* ^	224.1 (43.4)	26
“	“	45	606.15 (160.17)	ns	ns	* ^	223.4 (38.1)	26
“	“	60	834.45 (171.04)	ns	ns	* ^	203.9 (41.7)	20

<sup>1</sup> Confidence interval;

<sup>2</sup> Dunnnett's test , compared with each sole host (F = 22.12; d.f. = 26, 563;  $P \leq 0.05$ ) ; \* =significantly lower, \*^ = significantly higher, ns = non-significant.

Table 2. Mean percent ( $\pm$  SD) of *O. ovatus* larvae infected with *M. anisopliae* after 14 days exposure to soil inoculated at various rates. Means followed by the same letter are not significantly different ( $P < 0.05$ ).

Spores/g Dry Soil	% Larval Infection
$1 \times 10^7$	90 (7.0) a
$1 \times 10^6$	85 (6.5) a
$1 \times 10^5$	80 (7.1) a
$1 \times 10^4$	70 (2.5) a
Control	3 (2.5) b

### Figures

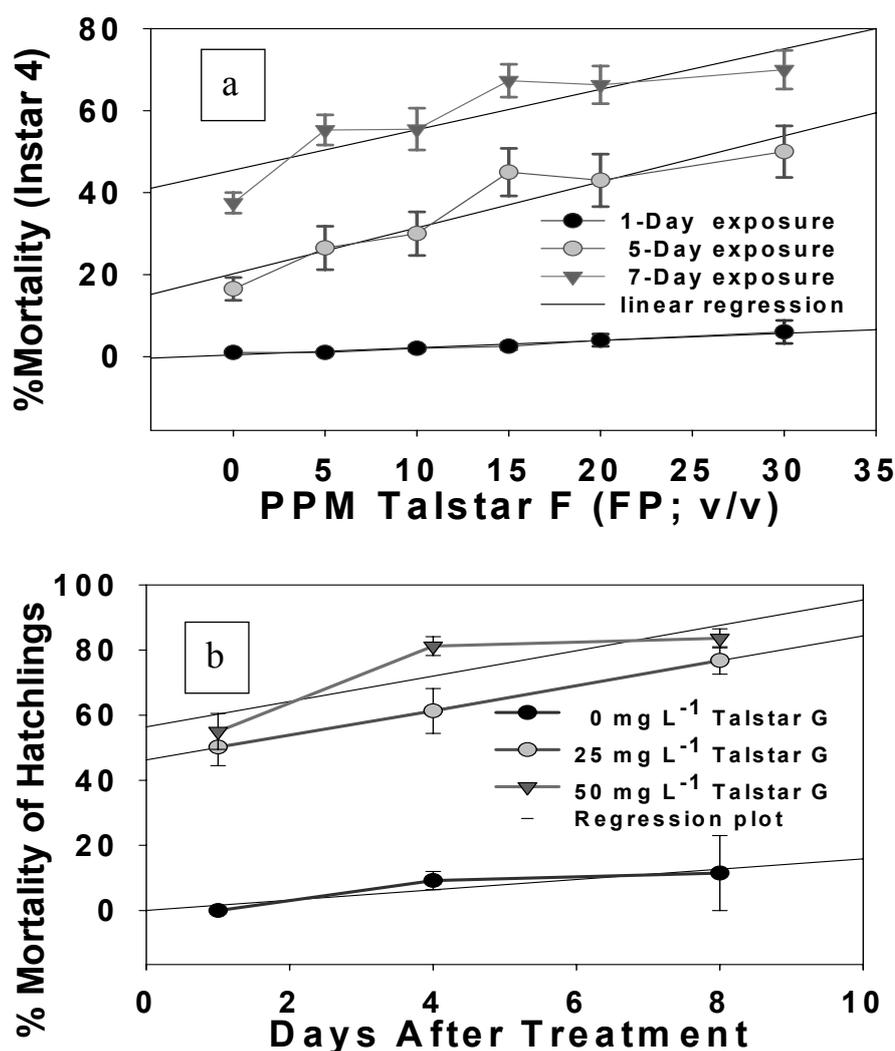


Fig. 1. a) Cumulative mortality of 4<sup>th</sup> instar *O. sulcatus* at Talstar F dilutions (0–30) mg L<sup>-1</sup> after 1, 5 or 7 days exposure in soil. b) Cumulative mortality of hatchlings of *O. sulcatus* at Talstar G dilutions (0, 25, 50 mg L<sup>-1</sup>) over 1, 4 and 8 days exposure in soil.

