Promotion of leaf degradation by earthworms under laboratory conditions

B. Heijne, A. de Jager, and P.F. de Jong

***Abstract* – Organic materials were applied to leaves from organic apple trees. Then, leaves were fed to earthworms in a laboratory culture. The objective was to select materials which promote leaf degradation by earthworms and consequently reduce the inoculum pressure of apple scab in orchards. Used earthworms were fully grown and consequently no effect of the leaf treatments was found on earthworm weights. However, leaf consumption tended to be increased by addition of amino acids and beet pulp to leaves. For beet pulp, this was especially the case when the dose was increased from 1 to 5 % or when it was freshly applied.[1]**

Introduction

Hibernation of *Venturia inaequalis*, the causal fungus of apple scab, occurs inside fallen leaves. Part of the leaves is still present in spring and from these leaves ascospores are produced to infect apples. Another part of the leaves is decomposed during winter time. Sanitation methods are developed to promote leaf decomposition and thereby reducing the amount of inoculum and facilitating apple scab control in spring.

The removal of leaves by earthworms is one of the factors involved in degradation of leaves with overwintering *Venturia inaequalis*. This part of the Repco-project is directed at stimulating their activity in order to reduce the amount of overwintering leaves as much as possible before bud break in spring. This study focuses on laboratory conditions of methods to increase activity of relevant earthworm species.

Earthworm species can be basically divided in different groups based upon feeding behaviour. Humic formers feed on (fresh) organic material like leaves or dung, and humic feeders consume large amounts of soil mixed with raw humus (Edwards and Bohlen, 1996). For use in laboratory studies, cultures were set up with the most important species with respect to consumption of apple leaves, *Lumbricus terrestris*, and *L. rubellus* both of them humic formers and relevant to the consumption of leaves.

Materials and methods

General lay-out of experiments

Three experiments were carried out with leaves collected from an organic orchard. These were air dried and stored prior to use. Leaves were regularly reshuffled to prevent rotting of leaves. Soil is collected from the organic field, dried and pasteurised. After drying the soil is sieved to remove big clods and known quantities of dry soil are carefully wetted with known quantities of tap water. Application is done with a watering can with fine droplets. All containers were then loosely filled with soil. Then, once carefully shaken. All individual earthworms are weighted and grouped into classes of similar weight. Five earthworms are placed on top of the soil of each container. Each container got 5 earthworms of similar weight composition; hence each container is exactly the same. Treated dry leaves are weighted in portions of about 10 grams. Subsequently, the leaves are carefully rewetted with tap water with a fine droplet, hand held sprayer. Every two to three days the conditions of soil and leaves is checked and rewetted if necessary to avoid drying out of soil and leaves. All leaves are replaced at the moment that about 80 % of the leaves were eaten. New leaves are prepared similar as described before and placed on top of the soil in the containers. The remaining leaf material is collected in paper bags, dried in a stove and weighted. Replacement of leaves is done as frequent as necessary to supply the earthworms with ample food.

All data were subjected to analyses of variance (ANOVA). Significant F-tests ($P < 0.05$) were followed by a Least Significant Differences test for pair wise comparisons of treatment means using $LSD_{0.05}$ values.

Treatments

Experimental treatments were application of materials to improve palatability and consumption by earth worms. All leaves were sulphur treated leaves obtained from the organic orchard at PPO, Randwijk, the Netherlands. All leaves were from cultivar Jonagold (*Malus x domestica* Borkh.). Materials tested were glucose 1 % or 5 %, beet pulp (Vinasse) 1 % or 5%, amino acids (Aminosol) 1 %, and as a reference urea 5 %. Standard sulphur-treated leaves were used as a control in these experiments.

Leaves were dipped in treatment solutions prior to feeding leaves to earthworms in the first experiment. Trees were sprayed with treatment solutions in the orchard just before leaf fall in the second experiment. Leaves were sampled from the treated trees and stored at 3 ºC in a cool room until use. In the third experiment, stored leaves were dipped in treatments solution similar to those in the first experiment. However, half of the treatments were

All authors are with Applied Plant Research (PPO-fruit), NL-6670 AE Zetten, The Netherlands (bart.heijne@wur.nl).

rewetted with the treatment solution, in stead of tap water.

Preparation of soil and containers

Soil was taken from an experimental organic orchard, at PPO, Randwijk, the Netherlands. The soils were managed as commercial arable field till 1995, and then lain fallow till 1999 when the orchard was established. The orchard was maintained according to standard organic schedule. The upper 5 cm of the soil was removed from the strip under trees. The soil was collected for the experiments from the layer from 5 to 30 cm below the original soil surface. Fresh soil was obtained from the same area for each experiment. The soil was dried and pasteurised at 70 ºC during 24 hours in a stove. Characteristics of the pasteurised soil, based on dry soil, were: organic content 2.4 %, pH (KCl) 7.3, MgO 249-255 mg/kg, K (HCl) 49 mg/100 g, carbonate 1.1-1.4 %, clay fraction 36-37 %. The water content was adjusted to 20 % a day before use. Pipe like containers were filled with the soil. Containers were made of PVC tubes with length and diameter of 25 and 15 cm respectively. The bottom of the tubes was sealed with nylon netting with square pores of 2 mm. The upper part was left open. Containers were filled with soil up to 5 cm below the rim of the container, hence containing a soil volume of 3534 cm^2.

RESULTS AND DISCUSSION

Earthworm weights

The majority of the *L. terrestris* earthworms were adult when using them in the experiments. It is not likely that they will, even under optimal conditions, gain much weight, because they were "full-grown". They might have produced cocoons, but we did not collect these from the soil in the containers. On top of that, we expect the conditions for *L. terrestris* were sub-optimal in our experiments. Under outdoor conditions *L. terrestris* lives in deep burrows (Butt, 1993). For practical reasons, our containers were only 25 cm height. These reasons might explain that there was little effect from the treatments on fresh body weight for this species.

Leaf consumption

Organic orchards are treated with sulphur to control apple scab. Leaf consumption by *L. terrestris* was determined of these sulphur-treated leaves and compared to non-sulphur treated leaves. There was little difference in leaf consumption. Therefore, all leaves used in the experiments were standard sulphur-treated leaves. These leaves were water-treated as a control treatment.

Table 1. Average leaf consumption (g) by *Lumbricus terrestris* either alone or in mixed culture with *Lumbricus rubellus* fed with differently treated apple leaves in experiment 1.

	Leaf consumption (g)			
Leaf treatment	Alone		Mixed	
water	24.48	b	27.27	B
urea 5 % + glucose 5 %	14.14	a	14.61	A
amino acids 1 %	28.48	c	30.49	C
beet pulp 5 %	27.60	c	28.44	bc

Table 2. Average leaf consumption (mg) by *Lumbricus terrestris* fed with differently treated apple leaves. Leaves were treated in the orchard and either or not additionally dipped before feeding in experiment 2. In experiment 3, leaves were once dipped and either or not rewetted with the same leaf treatment during the feeding period.

Leaf treatment	Leaf consumption (mg)			
	Experiment 2		Experiment 3	
water	2561	ab	2410	a
urea 5 % dip	796	a	2630	a
urea 5 %	2859	ab	-	
amino acids 1 % dip	5547	c	3753	b
amino acids 1 % rewetted	-		4501	b
amino acids 1 %	4911	c	-	
beet pulp 1 % dip	5290	c	2888	a
beet pulp 1 % rewetted	-		4188	b
beet pulp 1 %	2318	ab	-	
beet pulp 5 %	4477	bc	-	
glucose 1 % dip	-		4042	b
glucose 1 % rewetted	-		3802	b

Urea, applied freshly, had a negative effect on leaf consumption by *L. terrestris* in experiment 1 (Table 1). It is known (Curry, 1998) that ammonia is a repellent and even toxic to earthworms. This explains these negative effects, since ammonia comes free from urea treated leaves. Therefore, leaves were left outdoors for at least a few days to prevent this effect in the following experiments.

Both amino acids and beet pulp showed increased leaf consumption by *L. terrestris*, although not in all cases significantly. It was observed that the leaves became softer by those treatments, especially by those treated with beet pulp and earthworms showed good condition with a vivid appearance.

CONCLUSIONS

There is a tendency that amino acids and beet pulp promoted leaf consumption by the earthworm *Lumbricus terrestris*. For beet pulp, this was especially the case when the dose was increased from 1 to 5 % or when it was freshly applied.

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