

# Disintegration of Dung Pats from Cattle Treated with the Ivermectin Anthelmintic Bolus, or the Biocontrol Agent *Duddingtonia flagrans*

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**Dimander S-O, Höglund J, Waller P. J: Disintegration of dung pats from cattle treated with the ivermectin anthelmintic bolus, or the biocontrol agent *Duddingtonia flagrans*.** *Acta vet. scand.* 2003, 44, 171-180. – An experiment was performed during the grazing seasons of 1998, 1999 and 2000 to study the influence of the antiparasitic drug ivermectin and the nematophagous fungus *Duddingtonia flagrans* on cattle dung disintegration. The faeces originated from groups of animals that were part of a separate grazing experiment where different control strategies for nematode parasite infections were investigated. Each group consisted of 10 first-season grazing cattle that were either untreated, treated with the ivermectin sustained-release bolus, or fed chlamydo spores of *D. flagrans*. Faeces were collected monthly on 4 occasions and out of pooled faeces from each group, 4 artificial 1 kg dung pats were prepared and deposited on nylon mesh on an enclosed pasture and protected from birds. The position of the new set of pats was repeated throughout the 3 years of the study. Each year, the dung pats were weighed 4, 6, 8 and 10 weeks after deposition and immediately afterwards replaced to their initial positions.

Results showed that there was no difference in faecal pat disintegration between groups. However, the time-lag between deposition and complete disintegration of the faeces varied significantly between deposition occasions. Dung pats disappeared within 2 weeks (visual observation) when subjected to heavy rainfall early after deposition, whereas an extended dry period coincided with faeces still remaining 12 months after deposition.

*cattle; faecal pat; disintegration; ivermectin; biological control; Duddingtonia flagrans.*

## Introduction

There is probably no other habitat where so many organisms in such large numbers act simultaneously in the processes of biological decomposition, than in the dung of grazing livestock (Lodha 1974). However, there is also a vast number of abiotic factors, including pat size and shape, composition, moisture, pH and location as well as prevailing weather and mechanical disturbance, which influence the process of dung breakdown (Barth 1993). Obviously, the numerous variables involved in the

process of dung degradation and the differences in methodology and study designs require attention to detail in the interpretation and comparison of results between such studies (Barth 1993).

Avermectins are pesticides effective against a wide range of internal and external parasites of livestock (Campbell 1989) of which ivermectin was the first drug in this class to be marketed in 1981. Later in the mid 1990's, when ivermectin administered through a sustained-release device (bolus) was introduced, veterinarians and

farmers were provided with a highly effective tool for nematode parasite control of cattle. The ivermectin bolus is claimed by the manufacturer to release 12 mg ivermectin daily for 135 d and thus maintains the treated cattle virtually worm-free for most of the grazing season. However, concern has been raised about possible environmental and economic consequences when using avermectins (Edwards et al. 2001), particularly if administered through the sustained-release system (Herd et al. 1996, Wiktelius 1996). Clearly, certain developmental stages of some coprophilic invertebrates (dung beetles and flies) are particularly sensitive to ivermectin residues in ruminant dung (Strong 1993, Strong et al. 1996). A reasonable interpretation is that an absence of dung degrading organisms may account for a delay, or failure, in the disintegration of dung derived from bolus treated cattle (Wall & Strong 1987). Ivermectin could also have adverse effects on the soil dwelling nematode populations (Stretton et al. 1987, Jansson & Rabatin 1997). However, Barth et al. (1993) reported no significant difference in total numbers of soil nematodes in dung pats from cattle treated with the ivermectin bolus when compared with non-treated animals.

Recent research has shown that biological control of nematode parasites in livestock with the nematophagous fungus *Duddingtonia flagrans* may become part of an organically acceptable alternative (for review, see Larsen 2000). The resting spores (chlamydo spores) of the fungus are capable of surviving the gut passage in ruminants and with warm and moist conditions they germinate and spread in the faecal deposits. Parasite control is obtained by the trapping hyphal structures of this fungus, which have the ability to capture and destroy larval stages of the nematodes before they migrate to herbage and become available to grazing animals (for review, see Larsen 1999).

Saprophytic nematodes is an important and abundant taxonomic component of the grassland fauna (for review, see Bardgett et al. 1999) that rapidly colonise the fresh dung pats and play key roles in nutrient recycling (Yeates 1984, Ingham et al. 1985, Griffiths et al. 1995). Understandably, soil nematodes may also fall prey to *D. flagrans*.

Saprophytic soil nematode investigations have been conducted on pasturelands in Australia grazed by sheep fed *D. flagrans* (Yeates et al. 1997, Knox et al. 2002) and in Denmark (Faedo et al. 2002). In Sweden, two soil nematode studies have been performed on pasturelands grazed by cattle subjected to parasite control by the ivermectin sustained-release bolus and *D. flagrans* (Yeates et al. 2002, 2003). Both these studies were complementary to the cattle grazing experiment (Dimander et al. 2003) that served as source of faeces in the present trial. However, no investigation has yet been conducted on deployment of *D. flagrans* with respect to dung degradation.

The aim of the present 3-year plot trial was to compare the decomposition rate of uniform, artificial dung pats derived from 3 groups of cattle that were either treated with the ivermectin bolus, fed chlamydo spores of *D. flagrans*, or were maintained untreated.

## Materials and methods

### *Experimental pasture plots*

This study was conducted at the Kungsängen Research Centre, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden, between 1998 and 2000. The experimental site was a uniformly flat improved pasture, which consisted predominantly of smooth meadow grass (*Poa pratensis*) with smaller proportions of meadow fescue (*Festuca pratensis*), white clover (*Trifolium repens*), tussock grass (*Deschampsia caespitosa*) and couch grass (*Agropyron repens*). An enclosed area of 20×8 m was

designated for deposition of the faecal pats. Prior to deposition of the pats, the pasture was mowed to approximately 5 cm. The plot area was divided into 48 separate 80×80 cm sub-plots with 20 cm buffer zones between the 4 replicates. An extra 2 m buffer zone was created between treatments and deposition occasions. These buffer zones were mowed regularly to a sward height of approximately 2 cm.

#### *Experimental design and source of faeces*

Each year, faeces were obtained from 10 first-season grazing cattle per treatment group that were maintained on improved pastures at the Research Centre. These cattle were primarily part of a grazing experiment where alternative strategies for gastrointestinal nematode parasite control were investigated. The strategies studied were biological control using *D. flagrans*, grazing management with turnout on cow pasture in combination with mid-summer move to aftermath and treatment with a copper oxide wire particle bolus. These alternatives were compared with an untreated control group and cattle treated with the ivermectin sustained-release bolus (for details, see (Dimander *et al.* 2003). For the purpose of this study, the following treatments were included:

- Control: No anthelmintic or other medical treatment
- IVM: Ivermectin (1.72 g, 12 mg/d or 40-65 µg/kg bw/d for 135 d) intraruminal bolus (Ivomec SR vet., Merial, Paris, France) administered to each animal at turnout
- Fungus: *Duddingtonia flagrans* chlamydospores (Christian Hansen Biosystems A/S, Copenhagen, Denmark) administered daily for 90 d (d 21-111) mixed in concentrate (1×10<sup>6</sup> spores/kg body weight/d 1998; 0.5×10<sup>6</sup> spores/kg body weight/d 1999 and 2000)

During days 21 to 111 after turnout, all cattle

were daily fed a 1 kg grain supplement from troughs with 0.5 m space per animal.

Faeces were collected *per rectum* from all animals in each treatment 4, 8, 12 and 16 weeks after turnout in mid May each year, representing June, July, August and September depositions, respectively. From each of the groups, pooled faeces were thoroughly mixed and its dry matter (DM) determined based on 3 sub-samples of 10 g. From the mixed faeces for each treatment, 4 artificial 1.0 kg dung pats were prepared in aluminium pie dishes (21 cm Ø). The blocks of 4 pats per treatment and deposition occasion were assigned to sub-plots by random allocation the first year of the trial (1998). Each pat was deposited on stretchable, quadratic (approximately 25 cm) 8 mm nylon mesh in the middle of the sub-plots, marked and protected from disturbance by birds by covering with individual wide-meshed wire cages. Weighing of the faecal pats was performed 4, 6, 8 and 10 weeks after deposition by gently lifting the nylon mesh supporting the dung pat from the ground on to a field scale and then replacing as close as possible to the same alignment on the sub-plot. Visual examination of the plot area was performed daily for two weeks following deposition of each new set of faecal pats. The year-to-year pat location for faecal pats from the different treatments and deposition occasions was maintained during the following 2 years of the trial (1999 and 2000).

#### *Meteorology*

Precipitation and temperature data were recorded continuously at a meteorological station, located 2.5 km from the experimental site. Monthly precipitation and 10 d average temperature values were expressed in relation to the long-term (1961-1990) average (LTA) as shown in Figure 1.

In the summer of 1998, the weather conditions were wetter and cooler than the LTA. In 1999,

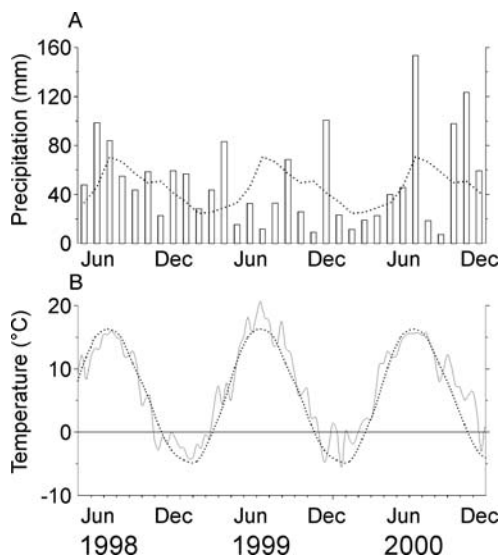


Figure 1. Meteorological data of the experimental site between May 1998 and December 2001. Comparisons between (A) the long-term (30-year) average (LTA) precipitation (dotted line) and the precipitation during the trial period (bars).

(B) LTA monthly mean temperature (dotted line) compared with 10-day mean temperature during the trial period (solid line).

the precipitation during the summer was exceptionally low while the temperature was above the LTA. In 2000, precipitation was above the LTA, whilst the temperature was normal. Daily mean temperature and precipitation during the sampling periods are shown in Figure 2.

#### Statistical analysis

Data were summarised using Microsoft Excel<sup>®</sup> 2000 and the statistical analysis was performed with Intercooled Stata 7.0 for Windows NT (Stata Corporation, College Station, Texas, USA). To obtain normal distribution and equal variances, weight of the dung pat was transformed to the 1.5 root prior to analysis according to the formula ( $\text{Weight}^{-1.5}$ ). Dung degrada-

tion was subsequently analysed in a repeated measurement ANOVA-model with treatment, year and month of deposition as independent variables, and weighing occasion as the repeated variable. The June 1998 and July 2000 depositions were excluded from the analysis as all dung pats for all treatments had disappeared at the time of the first weighing occasion. Dry matter content of the faeces was analysed using one-way ANOVA.

#### Results

The complete degradation time of 1 kg artificial dung pats varied between 2 weeks and 12 months in this experiment. No significant difference in dung degradation was detected between treatments during the 3-year study ( $p = 0.47$ ). However, differences were found between deposition months ( $p < 0.0001$ ) and years ( $p < 0.0001$ ) (Fig. 2). Faeces DM (Table 1) did not differ significantly between treatments ( $p = 0.10$ ).

In 1998, all pats deposited in June had vanished in less than 4 weeks. The pats deposited in July, August and September showed a similar pattern where approximately 400 g remained after 4 weeks and all visible faecal material had disappeared within 10 weeks after deposition.

In 1999, 14 mm of rainfall in 2 days after the June deposition prevented formation of a crust on the surface of the dung pats and extensive pat erosion was observed within 3 days. Rehydration due to 66.8 mm rainfall in 10 d in mid September caused an increase in weight of the remaining parts of the 1999 faecal pats deposited in June and July (week 10 sampling) and August (week 6 sampling). Remnants of all deposited faecal pats were still present 10 weeks after deposition and even in May 2000, small fragments of faeces (<20 g) remained on all sub-plots.

In 2000, the dung pats deposited in July had completely disappeared in less than 2 weeks

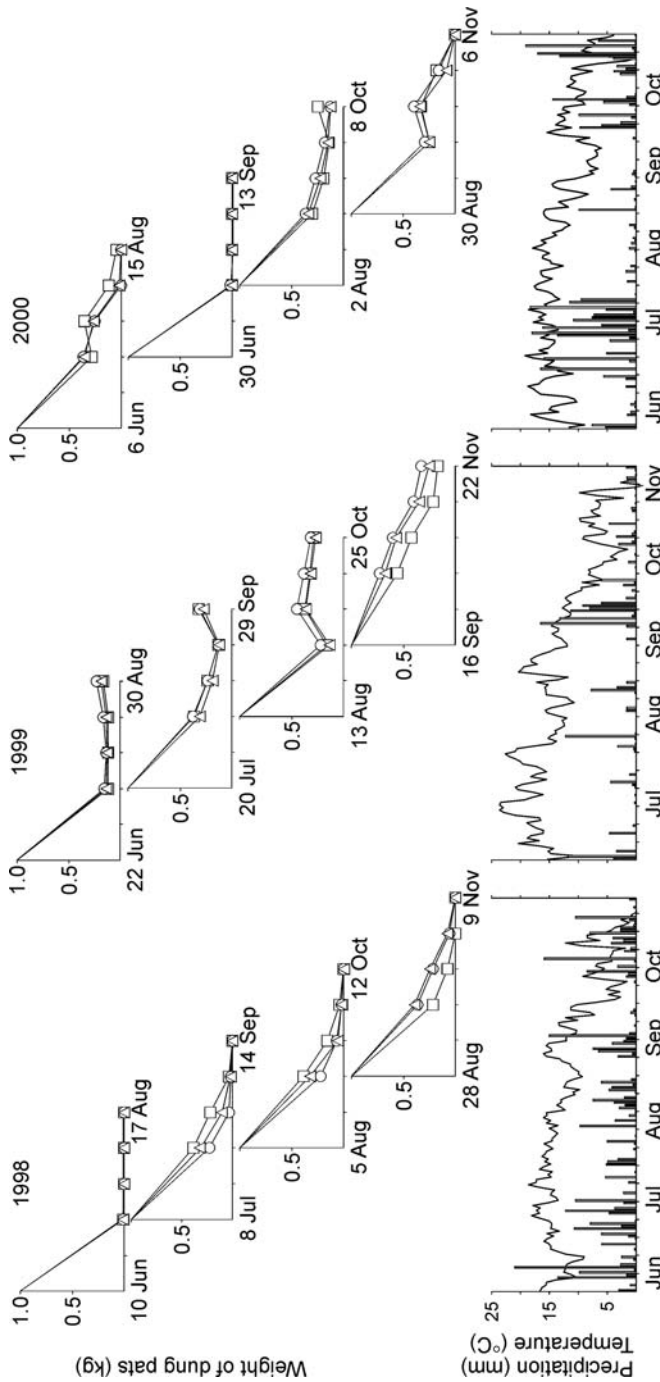


Figure 2. The change of mass of original artificial 1 kg dung pats derived from cattle that were either untreated, (Control; triangle), treated with the ivermectin bolus, (IVM Bolus; square), or fed the fungus *Duddingtonia flagrans* (Fungus; circle). The dung pats were protected from birds and deposited on a nylon mesh on 4 occasions during the grazing seasons 1998-2000, respectively. The year-to-year pat location for the different treatments and deposition occasions were repeated during the 3 years of the trial. Weighing of the pats was performed 4, 6, 8, and 10 weeks after deposition where the nylon mesh supporting the dung pat was lifted on to a scale and immediately afterwards replaced to its initial position. The corresponding daily mean temperature (solid line) and precipitation (bars) for the study periods are displayed below.

Table 1. Mean dry matter content (DM %) based on 3 sub-samples of 10 g of pooled faeces from untreated (Control), ivermectin bolus treated (IVM) and fungus treated (Fungus) first-season grazing cattle. Artificial 1 kg dung pats were prepared and deposited monthly between June and September during the grazing seasons of 1998, 1999 and 2000, respectively.

		1998			1999			2000		
		Control	IVM	Fungus	Control	IVM	Fungus	Control	IVM	Fungus
Jun	DM %	14	11	13	13	10	14	14	13	17
Jul	DM %	14	15	14	11	12	12	15	12	13
Aug	DM %	16	12	14	8	10	11	15	11	15
Sep	DM %	14	12	13	18	14	14	14	14	16

while approximately 300 g of the pats deposited in June, August and September remained 4 weeks after deposition. The dung pats deposited in June and September had all vanished 10 weeks after deposition whereas approximately 150 g of the pats deposited in August remained 10 weeks after deposition. However, no faeces remained in early May 2001.

### Discussion

Results from this 3-year experiment failed to show any significant differences in the disintegration rate of dung pats derived from cattle that were either treated with the ivermectin sustained-release bolus, or fed the nematophagous fungus *D. flagrans*, compared with untreated animals. However, significant differences in the faecal pat disappearance were detected between years and months of pat deposition. For example, the dung pats deposited in June 1998 and July 2000 disappeared in less than 2 weeks, while complete disappearance was prolonged for 9 to 12 months for the pats deposited throughout 1999. It is evident that these differences were attributed to variation in rainfall.

The formation of a crust on the surface of the cattle dung pat is of particular importance in the process of disintegration, and if this is prevented by continuous rainfall shortly after deposition, disintegration occurs more rapidly (Hypolite et al. 1984) as do simulation of con-

tinuously wet weather by irrigation (Dickinson et al. 1981). In contrast, a prolonged period of dry weather, which was a feature of summer and early autumn of 1999, extended the time-lag between dung pat deposition and complete disappearance for up to 12 months. Dry, sunny and hot weather conditions retard pat degradation (Dickinson et al. 1981, Anderson et al. 1984) due to the formation of an impervious hard outer crust that renders the pats relatively unattractive to coprophilic invertebrates and micro-organisms, as well as impeding their entry (Halley et al. 1993). Further, microbial and earthworm activity is slower during extended dry conditions (Halley et al. 1993).

In Scandinavia, dung beetles seem to play a minor role in the dung degrading process (Hanski & Cambefort 1991), whereas earthworms are more important (Holter 1977, 1979). The toxicity of ivermectin to the earthworm *Eisenia foetida* was investigated by Halley et al. (1989) who found that the 28 d LC<sub>50</sub> was 315 mg/kg soil. This is much higher than the 1.18 mg/kg faeces found by Alvinerie et al. (1999) in dung from cattle treated with the Ivomec SR bolus device (MSD AGVET, Paris, France), which is equivalent to the ivermectin bolus used in this trial. Indeed, no effect on lumbricoid earthworms of ivermectin in cattle dung was reported either by Wall & Strong (1987) or Madson et al. (1990). However, in contrast to these

findings are the observations in the laboratory by Gunn & Sadd (1994), who tested soil contaminated with a drug formulation containing 0.08% w/v ivermectin. Based on results from different concentrations of ivermectin in soil, the 14 d EC<sub>50</sub> for decreased growth rate of *E. fetida* was calculated to 4.7 mg/kg dry soil and the corresponding 14 d LC<sub>50</sub> for mortality was estimated to 15.8 mg/kg dry soil. Although the concentration in dung from animals treated with injectable or topical formulations may reach 16.3 mg/kg organic matter of dung (Sommer *et al.* 1992), this is still much higher than the 1.18 mg/kg faeces from ivermectin bolus treated animals (Alvinerie *et al.* 1999). Nevertheless, excreted ivermectin undergoes both oxidative degradation under aerobic conditions and rapid photodegradation if exposed to sunlight (Halley *et al.* 1993), which will diminish the environmental concentration of ivermectin over time. However, drug residues are excreted in faeces continuously for the period of 135 d when the bolus is active and are initially protected from sunlight within the pat (Halley *et al.* 1993).

For the first time, the rate of dung degradation of faeces produced by cattle fed *D. flagrans* was investigated. No statistical difference was found compared with faeces produced by untreated cattle. The trapping network is capable of capturing soil or dung inhabiting nematodes, which are in the order of 1-1.5 mm in length (Mankau 1962, Barron 1981). In a companion study to this work where herbage was clipped around the same faecal pats that was used in this study, *D. flagrans* significantly reduced herbage larval availability of gastrointestinal nematodes of cattle (Dimander *et al.* 2003). However, the nematode trapping activity of *D. flagrans* is not specific to parasitic larvae and the possible perturbation of the soil nematode community needs to be carefully examined. Two investigations in Sweden recently focussed

on this issue. These were trials conducted on pastureland grazed by the cattle that served as donors of faeces in this experiment (Yeates *et al.* 2002), as well as in a plot study where soil samples were taken directly underneath the dung pats of this experiment (Yeates *et al.* 2003). The results of these investigations showed no effect on total numbers, or diversity and functional groups, of soil nematodes. Similarly, in the study by Faedo *et al.* (2002), no statistical difference was found in the soil nematode population or the nematode composition fauna in soil surrounding sheep faeces. Moreover, Faedo *et al.* (2002) could not detect any spread of *D. flagrans* to the soil underneath the dung pats in the trial described by Yeates *et al.* (2003), neither did Knox *et al.* (2002) from soil profiles in Australia where sheep faeces containing *D. flagrans* had been deposited.

The short-term effect of *D. flagrans* on the lumbricoid earthworm *Aporrectodea longa* was examined by Grønbold *et al.* (2000). They found no indication of mycosis when exposed to cattle faeces during 20 d at a concentration of 800 chlamydo spores/g faeces. Additionally, the larger size earthworms compared with soil nematodes would prevent them from falling prey to *D. flagrans*.

Results from studies on dung degradation from ivermectin treated cattle in temperate regions are inconclusive. Herd (1995) reviewed the literature and identified factors and discussed possible reasons for the diversity of results. For example, Wall & Strong (1987) and Strong *et al.* (1996) reported that dung pats from cattle treated with the ivermectin sustained-release bolus failed to degrade normally and claimed that this was due to the toxic effects on some key dung-colonising insects. On the other hand, Wratten *et al.* (1993) and Barth *et al.* (1993) were unable to detect differences in the decomposition rate or in the organic matter content between dung pats from cattle treated with the

ivermectin bolus and those from untreated cattle. Indeed, the study by *Wratten et al.* (1993) was subject for critical analysis by *Holter et al.* (1994) where they attributed major experimental deficiencies for the absence of an effect on dung decomposition. Sound methodology in studies of dung degradation has been emphasised by *Barth* (1993) who pointed out the importance to measure moisture content of the dung in relation to dung pat disappearance. Similarly, when no retardation of dung degradation from ivermectin treated cattle was observed, *Strong* (1993) considered that this was due to flaws in methodology, statistics, and/or extremes of climatic conditions. Therefore, it is surprising that no data on moisture of dung were presented in the paper by the same authors (*Wall & Strong* 1987), which also makes interpretation of their results open to question.

In conclusion, excreted ivermectin, or *D. flammans*, at concentrations associated with specific nematode parasite control methods in cattle, had no significant effect on the rate of dung degradation in this experiment. As the aim of this study was to examine the change of dung mass only, the actual level of ivermectin in dung was not analysed and no specific investigation of the dung insect fauna was included. Consequently, the absence of a treatment effect does not exclude the possibility of an underlying detrimental effect on specific components of the dung insect fauna assemblage. However, prevailing weather conditions accounted for significant difference in dung degradation between deposition months and years and overruled any possible negative effect on the dung degrading fauna. In addition, these observations were consistent over 3 consecutive years and under both dry and wet weather conditions.

### Acknowledgements

We wish to acknowledge professor Arvid Ugglå for valuable comments on the manuscript, and competent technical assistance of Mr. Abdul Wahab Mehdi and Mss. Elisabeth Wilhelmsson and Maria Moberg. Supply of *Duddingtonia flagrans* spores by Christian Hansen Biosystems A/S and the Ivomec SR vet. bolus by Veter AB is gratefully acknowledged. The project was financially supported by the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Formas), contracts nos. 670.092/96 and 30.0214/99.

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## Svensk sammanfattning

*Nedbrytning av träck från kalvar behandlade med avmaskningsmedlet ivermektin eller rosvampen Duddingtonia flagrans.*

I föreliggande studie undersöktes nedbrytningshastigheten av komockor experimentellt. Under betessäsongerna 1998 till 2000 preparerades träck årligen vid 4 tillfällen från 3 grupper om vardera 10 första-gångsbetande kalvar. Kalvarna var antingen obehandlade, behandlade med en vomkapsel innehållande avmaskningsmedlet ivermektin eller utfodrades med sporer av rosvampen *Duddingtonia flagrans*. Fyra konstgjorda komockor tillverkades av en blandning träck från respektive djurgrupp och placerades på nylonnät på en betesyta som hölls fri från betesdjur och skyddade från fågelangrepp. Vägningar utfördes 4, 6, 8 och 10 veckor efter utplacering. Placeringen av komockorna för de olika behandlingarna och de olika deponeringstillfällena upprepades under de 3 åren.

Resultaten visade att ihållande regn i samband med, eller kort efter utplacering medförde att synlig träck försvann helt inom 2 veckor (visuell observation). I samband med långvarig torka fanns däremot rester av komockorna kvar upp till 12 månader efter deponering. Nedbrytningshastigheten påverkades dock inte av om träcken kom från kalvar behandlade med ivermektin eller rosvamp.

Studien visar att träcknedbrytningen framförallt påverkades av nederbörds mängd och nederbördsintensitet medan komockornas innehåll av ivermektin eller rosvamp inte påverkade nedbrytningshastigheten jämfört med mockorna preparerade från obehandlade kalvar. Detta utsluter emellertid inte att ivermektin eller *D. flagrans* kan inverka negativt på nedbrytningshastigheten av träck, men denna möjliga effekt överskuggades i föreliggande studie av meteorologiska faktorer.

(Received February 12, 2003; accepted September 23, 2003).

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