

# **Do herbivores facilitate seed germination and seedling recruitment of woody plants?**

**Tlou Julius Tjelele**

School of Life Sciences

College of Agriculture, Engineering and Science

University of KwaZulu-Natal

Pietermaritzburg

**2014**

**Do herbivores facilitate seed germination and seedling  
recruitment of woody plants?**

**Tlou Julius Tjelele**

**Submitted in partial fulfillment of the academic  
requirements for the degree of  
Doctor of Philosophy (Botany)**

School of Life Sciences  
College of Agriculture, Engineering and Science  
University of KwaZulu-Natal  
Pietermaritzburg

Supervisors: David Ward  
Luthando Dziba

**April 2014**

## **Preface**

The research results presented in this thesis were carried out at the School of Life Sciences, College of Agriculture, Engineering and Science of the University of KwaZulu-Natal at Pietermaritzburg campus, South Africa under the supervision of Prof. David Ward of the University of KwaZulu-Natal and Dr. Luthando Dziba of the Council for Scientific and Industrial Research (CSIR).

The thesis submitted for the degree of Doctor of Philosophy (PhD) in Botany has not been submitted in whole or in part to be examined any degree nor is it being submitted for any other degree at any University.

.....

Tlou Julius Tjelele (Candidate)

April 2014

Approved:

.....

Prof. David Ward (Supervisor)

April 2014

.....

Dr. Luthando E. Dziba (Co-supervisor)

April 2014

## **Declaration: Plagiarism**

I, Tlou Julius Tjelele, hereby declare that the results presented in this dissertation are my original work and that it has not previously in its entirety or in part been submitted at any university for a degree.

- This dissertation does not contain another person's writing unless acknowledged as sourced from other research. Where other sources have been quoted, then their words have been rewritten but the general information attributed to them has been referenced.
- This dissertation has not been submitted for any degree or examination at any other university.
- This dissertation does not contain text, graphics and/or tables from the internet, unless acknowledged and the sources included in the dissertation and in the reference section.

.....

Tlou Julius Tjelele (Candidate)

April 2014

College of Agriculture, Engineering and Science

## **Declaration 2: Publications**

The four chapter papers in this dissertation were written by Julius Tjelele, and David Ward and Luthando Dziba (supervisors) contributed valuable comments to the manuscripts. The chapter papers were published, submitted or prepared for different journals. The four chapter papers presented in this dissertation were prepared according to the requirements of the particular journal to which they were submitted or published.

### **Publication 1:** Published in *Rangeland Ecology and Management*

Tjelele TJ, Dziba LE and Pule HT. 2012. Recovery and germination of *Dichrostachys cinerea* seeds fed to goats (*Capra hircus*). *Rangeland Ecology and Management* 65: 105-108.

### **Publication 2:** Submitted to *Rangeland Ecology and Management*

Tjelele TJ, Ward D and Dziba LE. 2013. Effects of diet quality on germination of *Dichrostachys cinerea* and *Acacia nilotica* seeds fed to ruminants.

### **Publication 3:** Formatted for submission to *African Journal of Range and Forage Science*.

Tjelele TJ, Ward D and Dziba LE. 2013. The effects of gut passage and dung fertilization on seedling establishment of *Dichrostachys cinerea* and *Acacia nilotica*.

**Publication 4:** Formatted for submission to *Journal of Applied Ecology*. Tjelele TJ, Ward D and Dziba LE. 2013. The effects of livestock seed ingestion, dung fertilization, trampling, grass competition and fire on seedling recruitment of two woody plant species.

.....

Tlou Julius Tjelele (Candidate)

College of Agriculture, Engineering and Science

## **Dedication**

I would like to dedicate this dissertation to my wife Louisa Tjelele and daughters (Lethabo and Lerato Tjelele) for their patience during my studies.

To my late grandmother Manoko Lebogo who taught me the importance of discipline and perseverance to achieve major goals.

## Abstract

Woody plant encroachment is a phenomenon whereby trees and shrubs invade grasslands or increase in an already wooded area, resulting in lower yields of herbaceous plants and a reduction in the carrying capacity of rangelands. It is not only the extent of woody plant encroachment, but also the rate at which it occurs, that is a major concern for livestock ranchers interested in herbaceous production. The question of what causes woody plant encroachment still remains unanswered. Animals consume a considerable amount of woody plant seeds during the dry season and could be responsible for spreading undesirable species to new areas or they could enhance the dispersal of species already established. However, under natural conditions, seed dispersal does not guarantee seed germination, which depends on appropriate environmental and seed survival conditions. To better understand the mechanisms involved in woody plant encroachment, we studied the effects of livestock (goats, sheep and cattle), diet quality (high-quality (*Medicago sativa* hay) vs. low-quality (*Digtaria eriantha* hay)), seed characteristics (size, shape and hardness), grass competition and fire on germination and seedling recruitment of *Dichrostachys cinerea* and *Acacia nilotica* seeds. These objectives were achieved by conducting the following trials: a) recovery and germination of *D. cinerea* seeds fed to goats, b) diet quality on germination of *Dichrostachys cinerea* and *Acacia nilotica* seeds fed to ruminants, c) the effects of gut passage and dung fertilization on seedling establishment of *Dichrostachys cinerea* and *Acacia nilotica* seeds and d) the effects of gut passage, dung fertilization, trampling, grass competition and fire on seedling recruitment of the two woody plant species.

During the first trial, seed recovery rate of *D. cinerea* seeds consumed by goats, either voluntarily after mixing them with feed pellets (mixed), or by force-feeding (gavaged) and



germination percentage were measured. Seed recovery for the gavaged treatment (32.7%) was significantly higher than for the mixed treatments (9.9%;  $P < 0.001$ ). Seeds that passed through the digestive tract (mixed (35.5%)) and gavaged (31.2%) treatments had a significantly higher germination percentage than untreated seeds (19.0%). A non-negligible proportion of *D. cinerea* seeds remained intact after chewing and passage through the digestive system, and their germination percentage increased.

In the second trial, I tested the effects of animal species (goats and sheep, goats and cattle), diet quality (*Medicago sativa* hay and *Digitaria eriantha* hay), and seed characteristics (size, shape and hardness) on the effectiveness of animal seed recovery and germination of *D. cinerea* and *A. nilotica* seeds. The trial was divided into two experiments. In the first experiment, a significant interaction effect of animal species (goats, sheep), diet (high-quality hay, low-quality hay) and seed species (*A. nilotica* seeds, *D. cinerea* seeds) was found on percentage germination ( $P < 0.0001$ ). There was also a higher percentage seed recovery ( $P < 0.009$ ) when animals were offered high-quality hay ( $47.4\% \pm 4.65$ ) compared to low-quality hay ( $30.2\% \pm 3.24$ ). In goats fed *D. eriantha* hay, *A. nilotica* seed germination ( $9.38\% \pm 3.66$ ) was higher ( $P < 0.05$ ) than goats fed *D. eriantha* hay and *D. cinerea* seeds ( $6.78\% \pm 1.13$ ). A greater germination percentage was observed in goats fed *M. sativa* hay with *D. cinerea* seeds ( $6.71\% \pm 1.53$ ) than goats fed *M. sativa* hay with *A. nilotica* seeds ( $2.50\% \pm 0.97$ ) ( $P < 0.05$ ). In the second experiment, animal species had a positive impact, both on seed recovery ( $P < 0.0325$ ; goats  $32.0\% \pm 6.44$ ; cattle  $50.3\% \pm 4.27$ ) and germination percentage ( $P < 0.055$ ; goats  $14.1\% \pm 1.48$ ; cattle  $9.3\% \pm 0.94$ ; control: *D. cinerea*  $0.64 \pm 0.06$ ; *A. nilotica*  $0.59 \pm 0.07$ ). Animal species in experiment one (goats and sheep) and two (goats and cattle) was most important for seed recovery and germination. However, diet (*M. sativa* hay and *D. eriantha* hay) and seed species

(*D. cinerea*, *A. nilotica* seeds) also had important effects on germination of seeds retrieved from experiment one. The interaction of animal species and size, diet quality, and seed characteristics (size, hardness) all played a major role in recovery of viable and scarified seeds either alone or in combination.

I also studied the effects of seedling emergence, seedling establishment and recruitment of dispersed *A. nilotica* and *D. cinerea* seeds by goats and cattle under natural conditions. The interaction effect of animal species, seed recovery day and seed germination treatment/planting method was significant on seedling recruitment. Seeds retrieved from goats in the last four days and planted 2 cm in the soil with dung ( $25.85\% \pm 0.46$ ) and seeds planted 2 cm in the soil with no dung ( $24.77\% \pm 0.35$ ), recruited significantly better than seeds planted on top of the soil ( $16.98\% \pm 0.46$ ). The results also indicated significant differences in percentage seedling recruitment among goats, cattle and control, with goats and cattle having the highest percentage recruitment than controls or untreated seeds. Overall, seeds can potentially germinate and recruit following passage through the gut, thereby facilitating woody plant encroachment.

The results of the last trial showed that seed passage through the digestive tract of goats and cattle compared to untreated seeds (i.e. not ingested) played an important role in improving germination through seed scarification. However, seed recovery by livestock does not guarantee seedling establishment survival, survival and recruitment. Fire and grass mowing treatments affected seedling emergence, seedling survival and recruitment, most probably because of reduced grass competition, thereby reducing competition for resources (light, water and nutrients) between grasses and seedlings.

In conclusion, this study indicated that animal species (goats, sheep and cattle), associated diet (low-quality vs. high-quality), seed species (*D. cinerea* and *A. nilotica*) and seed characteristics (size, shape, hardness) all played an important role in seed germination. The interactions of animal species, fire, dung, and season either directly or indirectly were pivotal in the emergence, survival and recruitment of *D. cinerea* and *A. nilotica* seedlings. Thus, acid scarification in the gut of herbivores in combination with their indirect effects (dung fertilization) and removal of grass competition (either by fire or mowing) can facilitate seedling emergence, seedling survival and recruitment of woody plant species, which may lead to woody plant encroachment.

## **Acknowledgements**

No words can express how grateful I am to Prof. David Ward and Dr. Luthando Dziba for their leadership, patience, support, comments and suggestions throughout the study. I remember the first time I went with Dr. Dziba to the University of KwaZulu-Natal to present ideas of this work to Prof. Ward; it was the most difficult day of my life because I did not want to disappoint Dr. Dziba and also hoped that Prof. Ward would accept my ideas. Thanks to Prof. Ward for affording me an opportunity to work with you. I am also grateful to Dr. Dziba for the foundation he laid for me to become an independent thinker and shaping my science career. I have learned a lot as a scientist from both of you.

This study was made possible by the National Research Foundation (NRF) Thuthuka (PhD Track) Grant, Gauteng Department of Agriculture and Rural Development (GDARD), International Foundation for Science (IFS) Grant and Agricultural Sector Education Training Authority (AgriSETA) Grant. I would like to thank these institutions for the financial support to carry out the experiments, attend local and international conferences and purchase research equipment, all of which contributed to the successful completion of this study.

I would also like to thank the Agricultural Research Council-Animal Production Institute for providing research facilities in which all experiments were conducted. Data collection would not have been possible if it was not for the following people: Alan Short, Gilbert Pule, Michael Mokwala, Basanda Nondlazi, Marvin Mahvunga, Tebogo Matlou, Slindokuhle Khumalo, Lekukela Mohale, Thamsanqa Mpanza, Malenyalo Bathlatswi, Olga Mabelebele, Doreen Sithole, Basanda Nondlazi, Jan Manganye, Jerry Makondo and Andries Shokane. Each of these people contributed in different ways during different experiments. Your assistance is greatly

valued. I am also grateful to Ntuthuko Mkhize, also a PhD student who would listen and calm me during difficult times. A great appreciation to the Biometry Division of the Agricultural Research Council, specifically Frikkie Calitz and Eric Mathebula for their assistance with statistical analysis. I am thankful for their assistance and guidance.

I greatly thank my wife Louisa Tjelele for all the patience and support throughout the years as a student. It was very difficult for her when I would not be there for her and the kids, especially during data collection and writing-up. I will forever be grateful for all her support and endurance. I offer an apology to my wife and children for not being there when they needed me to accompany them for shopping and other important matters, and promise to devote time and energy to them post-PhD. I thank my parents (Johannes and Mosima Tjelele), especially my mother who consistently asked, even in my first year, when will I finish, that kept me on my toes. To my uncle Jacob Lebogo and my aunt Onicca Lebogo, thanks for the support and love you provided throughout my studies. Thanks to uncle Joseph Lebogo who advised me to study Agriculture post-matric.

Thanks to God for giving me strength and wisdom to complete this study and survive difficult times throughout my study.

## Table of Contents

Preface.....	iii
Declaration: Plagiarism.....	iv
Declaration 2: Publications.....	v
Dedication.....	vii
Abstract.....	viii
Acknowledgements.....	xii
List of Figures.....	19
Chapter 1.....	24
1.1. Introduction.....	24
1.1.1 Woody plant encroachment.....	24
1.1.2. Seed limitation.....	29
1.1.3. Establishment limitation.....	29
1.1.4. <i>Dichrostachys cinerea</i> and <i>Acacia nilotica</i> : common encroaching plant species in South African rangelands.....	30
1.1.5. Animal seed dispersal.....	31
1.1.6. Objectives.....	32

Paper chapters in this study are published, submitted and prepared for different journals. Hence they are formatted differently. A certain amount of repetition (especially of description of study area) is inevitable. .... 34

1.2. References..... 34

2. Chapter 2..... 47

Recovery and germination of *Dichrostachys cinerea* seeds fed to goats (*Capra hircus*) ..... 47

2.1. Abstract ..... 48

2.2. Introduction..... 49

2.3. Materials and methods ..... 50

2.4. Results and Discussion ..... 53

2.5. Management Implications..... 55

2.6. Acknowledgements..... 55

2.7. References..... 56

2.8. Figure Legend ..... 60

Chapter 3..... 63

Effects of diet quality on germination of *Dichrostachys cinerea* and *Acacia nilotica* seeds fed to ruminants ..... 63

3.1. Abstract .....	64
3.2. Introduction.....	66
3.3. Materials and methods .....	68
3.4. Results.....	72
3.5. Discussion .....	75
3.6. Management Implications.....	78
3.7. Acknowledgements.....	78
3.8. Literature Cited .....	79
3.9. Figure Legends .....	85
Chapter 4.....	89
The effects of gut passage and dung fertilization on seedling establishment of <i>Dichrostachys cinerea</i> and <i>Acacia nilotica</i> .....	89
4.1. Abstract .....	90
4.2. Introduction.....	91
4.3. Materials and methods .....	94
4.4. Results.....	97
4.5. Discussion .....	103



4.6. Conclusions.....	106
4.7. Acknowledgements.....	106
4.8. References.....	107
4.9. Figure Legends .....	117
Chapter 5.....	124
The effects of seed ingestion by livestock, dung fertilization, trampling, grass competition and fire on seedling establishment of two woody plant species .....	124
5.1. Summary.....	125
5.2. Introduction.....	126
5.3. Materials and methods.....	129
5.4. Results.....	133
5.5. Discussion .....	136
5.6. Conclusion .....	139
5.7. Acknowledgements.....	140
5.8. References.....	141
5.9. Figure Legends .....	154

Chapter 6.....	161
6.1. Synthesis .....	161
6.2. Management implications.....	166
6.3. Future research.....	167
6.4. References.....	170

## List of Figures

**2.1 Figure 1:** Cumulative recovery (as % of seed consumed) of *D. cinerea* seeds from goats fed using two methods (mixed seed and gavaged seed).

**2.2 Figure 2:** Cumulative seed germination percentage among four treatments: mixed seed, gavaged seed, mechanically scarified seed, and control (untreated) seeds over three periods (days 0-4, 5-10 and 11-16).

**3.1 Figure 2:** Significant effect of diet (high-quality hay and low-quality hay) on mean cumulative percentage seed recovery from experiment one. Bars represent standard errors (S.E).

**3.2 Figure 2:** Significant effect of animal species on mean cumulative percentage seed recovery from experiment two. Bars represent standard errors (S.E).

**4.1 Figure 1:** Mean monthly precipitation and minimum and maximum temperatures for 2011-2012 at the Agricultural Research Council's Roodeplaat Experimental Farm.

**4.2 Figure 2:** Significant interaction effect of animal species (cattle and goats), seed species (*D. cinerea* and *A. nilotica*) and seed recovery day on mean cumulative percentage seed recovery. Error bars represent standard errors (SE). Fisher's *post hoc* test was used.

**4.3 Figure 3:** The interaction effect on seedling emergence of animal species (goats, cattle) and unpassed/untreated seeds (i.e. not ingested), seed recovery day (first three days (F3)), last four days (L4)) and planting methods (seeds placed on top of the soil with no dung (Top), seeds buried 2 cm in the soil with no dung (ND), seeds buried 2 cm in the soil with dung (WD). Bars represent standard errors (S.E). Fisher's *post hoc* test was used.

**4.4 Figure 4:** The interaction effect on seedling survival of animal species (goats, cattle) and unpassed/untreated seeds (i.e. not ingested), seed recovery day (first three days (F3)), last four days (L4)) and planting methods (seeds placed on top of the soil with no dung (Top), seeds buried 2 cm in the soil with no dung (ND), seeds buried 2 cm in the soil with dung (WD). Bars represent standard errors (S.E). Fisher's *post hoc* test was used.

**4.5 Figure 5:** The interaction effect on seedling recruitment of animal species (goats, cattle) and unpassed/untreated seeds (i.e. not ingested), seed recovery day (first three days (F3)), last four days (L4)) and planting methods (seeds placed on top of the soil with no dung (Top), seeds buried 2 cm in the soil with no dung (ND), seeds buried 2 cm in the soil with dung (WD). Bars represent standard errors (S.E). Fisher's *post hoc* test was used.

**5.1 Figure 1:** The interaction effects of animal species (goats, cattle), unpassed/untreated seeds (i.e. not ingested), grass (G) (and mowed grass (Gc)), fire (F) (and no fire (Fc)) on seedling recruitment during the dry season. Bars represent standard errors (S.E). Same letters on the bars mean  $P > 0.05$ . Fisher's *post hoc* test was used.

**5.2 Figure 2:** The interaction effects of animal species (goats, cattle), unpassed/untreated seeds (i.e. not ingested), dung (D) (and no dung (Dc)), and grass (G) (and mowed grass (Gc)) on seedling recruitment during the dry season. Bars represent standard errors (S.E). Same letters on the bars mean  $P > 0.05$ . Fisher's *post hoc* test was used.

**5.3 Figure 3:** The interaction effects of animal species (goats, cattle), unpassed/untreated seeds (i.e. not ingested), seed species (*A. nilotica*, *D. cinerea*), grass (G) (and mowed grass (Gc)), fire (F) (and no fire (Fc)) on seedling emergence during the dry season. Bars represent standard errors (S.E). Same letters on the bars mean  $P > 0.05$ . Fisher's *post hoc* test was used.

**5.4 Figure 4:** The interaction effects of seed species (*A. nilotica*, *D. cinerea*), dung (D) (and no dung (Dc)), fire (F) (and no fire (Fc)) on seedling survival during the dry season. Bars represent standard errors (S.E). Same letters on the bars mean  $P > 0.05$ . Fisher's *post hoc* test was used.

**5.5 Figure 5:** The interaction effects of seed species (*A. nilotica*, *D. cinerea*), dung (D) (and no dung (Dc)), grass (G) (and mowed grass (Gc)) on seedling emergence during the wet season. Bars represent standard errors (S.E). Same letters on the bars mean  $P > 0.05$ . Fisher's *post hoc* test was used.

## List of Tables

**3.1 Table 1.** Interaction effects of animal species (goats, sheep), diet (grass hay, alfalfa hay) and seed species (*A. nilotica*, *D. cinerea*) on mean  $\pm$  S.E percentage seed germination.

**4.1 Table 1.** *F* values and *P* values for the effects of the four treatments and their interactions on seed germination, seedling survival and seedling recruitment.

**5.1 Table 1:** The seedling emergence trial consisted of a completely randomized design with three replicates per treatment: passage through goats or cattle or unpassed/untreated seeds, dung, grass competition, fire and trampling.

**5.2 Table 2:** *F* values and *P* values for the effects of the six treatments and their interactions and their interactions on seedling emergence, seedling survival and seedling recruitment. Dashes (-) = non-significant.

# Chapter 1

## 1.1. Introduction

Savannas are communities or landscapes with a continuous herbaceous layer, usually dominated by grasses, herbs and forbs as well as trees and shrubs that constitute an important source of food for wild and domestic animals (Frost et al. 1986, Scholes and Archer 1997). Savannas cover about 34% of South Africa, 54% of southern Africa and 60% of sub-Saharan Africa and support a significant proportion of the world's population (Scholes and Archer 1997). However, human disturbances have led to extensive modification of savannas through suppression of and deterioration of range condition caused by heavy grazing, wood harvesting for fuel, plant invasion caused by changes in fire regime and global climate change (Scholes and Archer 1997, Smit et al. 1999, Bond et al. 2003, Ward 2010).

### 1.1.1 Woody plant encroachment

Woody plant encroachment has been reported throughout the savannas of southern Africa (van Vegten 1983, Skarpe 1986, Smit 2004). Woody plant encroachment is a phenomenon where trees and shrubs invade grassland and/or increase in density, resulting in lower yield of herbaceous plants (Trollope 1980, Skarpe 1990, Richter et al. 2001, Ward 2005). The grazing capacity of large areas of southern Africa has declined because of the extent of woody plant encroachment (Grover and Musck 1990, Milton and Dean 1995, Scholes and Archer 1997, Smit 2004, Ward 2005). This poses a major challenge to land users because it impacts negatively on the herbaceous layer, which provides a substantial proportion of forage for livestock production (Lacey et al. 1992, Wigley et al. 2010).



Woody plant encroachment has a major negative effect on rangelands by reducing carrying capacity for livestock and increasing costs associated with livestock management (Archer 1990, Hagos and Smit 2005, Ward 2005, Kgosikoma et al. 2012). Thus, woody plant encroachment can be regarded as a form of land degradation because an increase, in the extent and density of woody plants, occurs at the expense of desirable grasses and forbs (Archer and Pyke 1991, Lacey et al. 1992, Ward 2005, Scholes 2009). Although there have been numerous studies on woody plant encroachment (van Vegten 1983, Milton and Dean 1995, O'Connor 1996, Jacobs 2000; Roques et al. 2001, Brits et al. 2002, Smit 2004), these studies have not yielded a broad understanding of the problem and an integrated approach to manage increasing woody plant dominance (Ward 2005, Wiegand et al. 2005, 2006).

Factors such as global climate change, rainfall variability, soil nutrients, herbivory and fire may also contribute towards increasing woody plant dominance (Frost et al. 1986, Skarpe 1992, Bond 2008, Ward 2010). The interaction of rainfall, soil nutrients, herbivory and fire largely determines the tree-grass ratio (Kraaij and Ward 2006). Conventionally, factors affecting the tree-grass ratio are explained by the competitive ability of grasses in acquiring resources, e.g. water, soil nutrients and fire (Walker et al. 1981, Noy-Meir 1982, Stuart-Hill and Tainton 1989, Davies et al. 1998, Sankaran et al. 2004, Ward and Esler 2010). The two-layer soil water hypothesis (Walter 1939) assumes that water is the primary limiting factor, where grasses are superior competitors for water in the surface soil layer than trees and shrubs which can utilize deeper soil resources (Walker et al. 1981, Walker and Noy-Meir 1982, Ward et al. 2013). When grasses are removed as a result of heavy grazing by livestock or fire, grasses absorb less water, which then becomes available for trees (Noy-Meir 1982). Furthermore, when woody plants germinate, grasses will have a competitive advantage but the situation is reversed once the

woody plant seedlings reach a certain size (van Auken and Bush 1989, Cramer et al. 2007, Ward and Esler 2010). Grass competition usually reduces the rate of change of grassland or open savanna to dense woodlands but does not prevent the eventual change (van Auken 2009).

Mechanisms permitting trees and grasses to co-exist, and factors determining the relative proportion of woody and herbaceous components in different savanna types remain unclear (Scholes and Archer 1997, Higgins et al. 2000). Despite several discussions on savanna dynamics, there is an understanding that the factors leading to the long-term coexistence of trees and grasses vary among different savannas (Jeltsch et al. 2000, Lehmann et al. 2011). Savannas have been interpreted in the context of equilibrium, non-equilibrium and disequilibrium dynamics (Illius and O'Connor 1999, Higgins et al. 2000, Sullivan and Rohde 2002). Equilibrium mechanisms refer to savannas where tree-grass co-existence is not dependent on rainfall variation or disturbances such as fire and herbivory (Scholes and Archer 1997). Niche separation by phenology has been proposed as a mechanism that can contribute to equilibrium forms of tree-grass co-existence in savannas (Scholes and Archer 1997). Savanna trees are able to store water and nutrients which enable them to achieve full leaf expansion prior to, or within a few weeks following the rains (Scholes and Archer 1997). Equilibrium co-existence arises because a superior competitor (grasses) becomes self-limiting at a biomass insufficient to exclude the inferior competitor, trees (Scholes and Archer 1997, House et al. 2003, van Langevelde et al. 2003, Sankaran et al. 2004). Non-equilibrium mechanisms refer to savannas where tree-grass co-existence is dependent on inter-annual rainfall variability and disturbances such as fire and grazing that switch the competitive balance between trees and grasses and/or provide opportunities for tree germination and establishment (Sankaran et al. 2004). This promotes the coexistence of both life forms in the system and results in a more dynamic tree-

grass ratio. Disequilibrium mechanisms refer to savannas as an unstable state and its existence is due to disturbances, which maintain the tree-grass coexistence preventing the complete dominance of trees or grasses (Sankaran et al. 2004, Accatino et al. 2010). Within the disequilibrium paradigm, the tree-grass coexistence has been explained through the demographic bottleneck mechanisms (see Sankaran et al. 2004).

It is clear that many interacting factors at various spatial and temporal scales contribute to create and maintain savannas (Rebertus and Burns 1997). The mechanisms and processes responsible for savanna existence occur when the system is driven to its boundaries (Jeltsch et al. 2000). In addition, mechanisms which prevent the savanna system from switching to another type of ecosystem (e.g. grassland and forest) are referred to as ecological buffering mechanisms (Jeltsch et al. 2000). Examples of buffering mechanisms in different savannas include: fire, browsers and microsites favoring tree establishment and survival (Jeltsch et al. 2000). Fire is the most prominent buffering mechanism, which often inhibits the transition to woodland or forest by destroying the juvenile trees and shrubs (Trollope 1982). Despite the importance of fire in savannas, the effect may differ depending on frequency, time of burning and the intensity of fire (Trollope 1980, 1982). In mesic areas, fire is usually common and intense after good rain as a result of high grass biomass production (Skarpe 1991). Fire-dependent plant species exhibit increased seedling recruitment following fire (Zammit and Westoby 1987), which lead to encroachment (Ward 2005). Dense woody plants result in a reduced shrub and herbaceous layer and, therefore, less grass fuel that reduces fire intensity (Trollope 1980, Richter et al. 2001, Bodmer and Ward 2006).

Browsing alone or in combination with fire may be an important factor impeding the transition of savanna vegetation to woodland because browsers reduce growth and regeneration

success of woody species (Cumming 1982, McNaughton 1992, Skarpe 1992, Jeltsch et al. 2000). The other important factor buffering the savanna transition to grassland is probably the availability of microsites with favorable conditions for tree seedling establishment and survival (O'Connor 1995, Fuhlendorf 1999, Jeltsch et al. 2000). Suitable microsites enable tree seedling establishment despite the competition for resources with the grass layer (Jeltsch et al. 1996, Cramer et al. 2007, Grellier et al. 2012). Another type of microsite that may play an important role in tree seedling establishment is tree-seed patches in herbivore dung (Jeltsch et al. 2000). For instance, consumption of *Acacia* seed pods by herbivores often leads to seed accumulations in the dung patches away from the mature trees and improved establishment conditions (Reid and Ellis 1995, Jeltsch et al. 1998). During low rainfall periods, intra- and interspecific competition for moisture reduces the chances of tree seedling establishment near mature trees and “additional establishment patches” away from mature tree populations (Jeltsch et al. 1998, 1999, 2000).

Woody plant encroachment is too complex to be associated solely with the two-layer hypothesis (Ward 2005, Wiegand et al. 2005, 2006, Ward et al. 2013). This is because a combination of factors, that are difficult to disentangle, act together to influence tree-grass co-existence (van Auken 2009). Among other factors, animal seed dispersal, plant chemical defences, fire and climate change may contribute to an increase in woody plant encroachment (Trollope 1980, Gardener et al. 1993, O'Connor 1996, Rohner and Ward 1999, Sankaran et al. 2004, Tews et al. 2004).

Seed dispersal, seed germination, seedling establishment and seedling recruitment are pivotal in plant population dynamics because of their influence on the distribution and abundance of woody plant species (Salazar 2010). The appearance of a radicle marks the end of

seed germination and the beginning of seedling establishment, a period that ends when the seedling has exhausted the food reserves stored in the seed (Westoby et al. 1996, Weitbrecht et al. 2011). Seedling recruitment refers to the process by which new individuals/seedlings establish in a new population or are added to an existing population (Herrera et al. 1994, Schupp and Fuentes 1995). Seedling recruitment was determined from the difference between the number of germinated seeds and the number of seedlings that died since the beginning of the experiment (see also Grellier et al. 2012). Successful seedling establishment must overcome two factors, i.e. seed limitation and establishment limitation.

### **1.1.2. Seed limitation**

Seed production and dispersal are vital processes in population dynamics, mainly because not all sites are reached by available seeds (Salazar 2010). Seed limitation refers to the failure of seeds to arrive at a suitable site (Ericksson and Ehrlén 1992, Nathan and Muller-Landau 2000, Turnbull et al. 2000). Seed limitation can arise from limited seed numbers and/or limited seed dispersal. Thus, if there is seed limitation, there will be few seedlings, which in turn are dependent on establishment conditions (Clark et al. 2007). Post-dispersal processes such as seed predation, e.g. predation by bruchid beetles (Lamprey et al. 1974, Rohner and Ward 1999, Bodmer and Ward 2006, Ward et al. 2010, Rodriguez-Perez et al. 2011) and loss of seed viability may constrain seedling recruitment by reducing the number of seeds available for germination (Campbell and Clarke 2006).

### **1.1.3. Establishment limitation**

Establishment limitation is the absence of seedling recruitment because of limited availability of suitable sites for seeds to germinate and establish (Clark et al. 1998; Muller-Landau et al. 2002).

Seedlings are vulnerable to desiccation from soil drying, which often limits root extension (Lambers et al. 1998). In addition, insufficient light, rainfall, nutrient availability, abundant grass biomass and predation constrain seed germination and seedling establishment of woody plants (O'Connor 1995, Hoffmann 1996, Kraaij and Ward 2006, Ward and Esler 2010).

#### **1.1.4. *Dichrostachys cinerea* and *Acacia nilotica*: common encroaching plant species in South African rangelands**

*Dichrostachys cinerea* (sickle-bush) and *Acacia nilotica* (scented-pod thorn) are very common encroaching woody plant species in South African rangelands (van Vegten 1983, Smit 2004). *D. cinerea* originated in Africa and has spread to many tropical areas in Asia and Australia (Coates-Palgrave 2002). *D. cinerea* is a thorny, semi-deciduous leguminous shrub with sizes ranging from 3-7 m high; it is generally found in frost-free areas in poor soils, fallows and degraded lands (Coates-Palgrave 2002). The species has an open round crown, a deep tap root and many lateral roots that makes eradication difficult (Coates-Palgrave 2002). *D. cinerea* trees have a cluster of pods that are twisted and indehiscent and fall from the tree between May and September. The pods are relatively high in protein (18.0%), and are therefore preferred by wild and domestic ruminants during the periods of food scarcity (Mlambo et al. 2004, Aganga and Motshewa 2007). Goats have been used to control encroaching woody plant species e.g. *D. cinerea*, yet their role and that of other ruminants on recovery of viable seeds are unknown (Chapter 2 and Chapter 3).

*Acacia nilotica* is naturally widespread in the drier areas of Africa and Asia (Bargali and Bargali 2009). The species grows to 15-18 m in height with reddish brown and smooth bark when young but becoming blackish and rough with age (Coates-Palgrave 2002, Bargali and

Bargali 2009). Pods are 8-17 cm long, and indehiscent. *A. nilotica* pods have a sweet smell and ripen from March to September (Coates-Palgrave 2002). *A. nilotica* seeds may still germinate 15 years after seed drop (Bargali and Bargali 2009). However, unless the disseminated seeds germinate in a relatively short period, they may never add to the recruitment of woody plants. The seeds of many savanna woody trees including *D. cinerea* and *A. nilotica* are subjected to a high incidence of predation, especially by bruchid beetles (Miller and Coe 1993, Miller 1994a, 1996), which could reduce the chances of germinating.

#### **1.1.5. Animal seed dispersal**

The pods and fruits of different woody plant species form an important part of the diet of browsers and grazers during the dry season (Coe and Coe 1987, Or and Ward 2004, van Auken 2009). Some woody plants have a hard seed coat, which enables ingested seeds to pass out undamaged from the faeces (Or and Ward 2004). Consumed seeds often require scarification, which may occur during mastication and passage through the digestive tract of animals, in order to germinate faster than normal seeds (Bodmer and Ward 2006). This, in turn, may result in increased seed dispersal, establishment and recruitment of woody plant seedlings. Seed dispersal through faeces by herbivorous animals may contribute to an increase in woody plant encroachment (Gardener et al. 1993, O'Connor 1996, Rohner and Ward 1997, Tews et al. 2004). The influence of browsing or grazing animals on seed dispersal, germination, establishment and recruitment of woody plants remains unclear because there are conflicting results from various studies (Janzen 1984, Rohner and Ward 1999, Barnes 2001, Materechera and Materechera 2001). Some studies have reported that passage through the digestive tract of herbivores enhances germination rates (Materechera and Materechera 2001, Bodmer and Ward 2006), while others have reported zero germination rates (Janzen 1984, Barnes 2001), although zero to low

germination may be induced by bruchid-infested seeds (Or and Ward 2004). Grellier et al. (2012) found no effect of gut passage or dung on *Acacia sieberiana* germination and recruitment. Herbivores may remove seeds from the tree prior to infestation or at least prior to reinfestation (Rohner and Ward 1999, Or and Ward 2003). However, Rohner and Ward (1999) found that bruchids infest many seeds while still on the trees and also seeds that are on the ground. This last-mentioned result indicates that mammals may not control infestation levels. The damage caused to *Acacia* seeds by bruchid beetles depends primarily on larvae and secondarily on the proportion of cotyledons eaten (Lamprey et al. 1974, Rohner and Ward 1999, Bodmer and Ward 2006, Ward et al. 2010, Rodriguez-Perez et al. 2011). The larvae of bruchid beetles enter the seeds by drilling in and feeding on the embryo and endosperm, leaving most of the infested seeds non-viable and negatively affecting seedling vigour for those that remain viable (Al Jabr Ahmed 2008, Rohner and Ward 1999, Bodmer and Ward 2006). To this end, animal seed dispersal, seed germination and seedling survival are critically important in understanding woody plant encroachment.

#### **1.1.6. Objectives**

The overarching objective of this study was to understand how seed recovery by browsing/grazing animals might contribute to the increase of woody plant encroachment (Cain et al. 1998). In savannas, fire and browsing have been used as tools to manage woody plant encroachment (El Aich and Waterhouse 1999). Nonetheless, the role of browsing/grazing animals in dispersal of woody plant seeds, seedling emergence, seedling establishment and recruitment remain little known and a major concern for land users.



The main objectives of this study are to:

- 1) determine the factors that underlie woody plant encroachment, specifically on seed recovery from grazing/browsing animals,
- 2) investigate the influence of grazing/browsing animals on seed recovery, germination potential, establishment and seedling recruitment of woody plants,
- 3) explore the effects of gut passage, grass competition and fire on seedling recruitment of woody plant species, and
- 4) explore appropriate management strategies and make recommendations.

These objectives will be achieved by conducting the following experiments designed to improve understanding of: **a)** recovery and germination potential of *Dichrostachys cinerea* seeds fed to goats (*Capra hircus*), **b)** the effect of diet quality on germination of *Dichrostachys cinerea* and *Acacia nilotica* seeds fed to ruminants, **c)** the effects of gut passage and dung fertilization on seedling recruitment of *Dichrostachys cinerea* and *Acacia nilotica* seeds, and **d)** the effects of seed ingestion by livestock, dung fertilization, trampling, grass competition and fire on seedling recruitment of two woody plant species.

Overall, this study is envisaged to contribute to a greater understanding of some factors that underlie woody plant encroachment, specifically the effects of seed recovery from grazing or browsing animals.

Paper chapters in this study are published, submitted and prepared for different journals. Hence they are formatted differently. A certain amount of repetition (especially of description of study area) is inevitable.

## 1.2. References

- Accatino F, De Michele C, Vezzoli R, Donzelli D, Scholes RJ. 2010. Tree-grass co-existence in savanna: interactions of rain and fire. *Journal of Theoretical Biology* 267: 235-242.
- Aganga AA, Motshewa CB. 2007. Nutritive value of urea molasses block containing *Acacia erubescens* or *Dichrostachys cinerea* as natural protein source. *Journal of Animal and Veterinary Advances* 6: 1280-1283.
- Al Jabr Ahmed, M. 2008. Effect of bruchid beetles infestation on the germination of *Acacia tortilis* seeds. *American Journal of Environmental Science* 46: 285-288.
- Archer S. 1990. Development and stability of grass/woody mosaics in a subtropical savanna parkland, Texas. U.S.A. *Journal of Biogeography* 17: 453-462.
- Archer S, Pyke D. 1991. Plant-animal interactions affecting plant establishment and persistence on revegetated rangeland. *Journal of Range Management* 44: 558-565.
- Bargali K, Bargali SS. 2009. *Acacia nilotica*: a multipurpose leguminous plant. *Nature and Science* 7: 11-19.
- Barnes ME. 2001. Seed predation, germination and seedling establishment of *Acacia erioloba* in northern Botswana. *Journal of Arid Environments* 49: 541-554.

- Bodmer RE, Ward D. 2006. Frugivory in large mammalian herbivores. In: Danell K, Duncan P, Bergstrom R, Pastor J (eds), *Large Herbivore Ecology, Ecosystem Dynamics and Conservation*. Cambridge University Press, Cambridge, UK. pp 232-260.
- Bond WJ, Midgley GF, Woodward WI. 2003. The importance of low atmospheric CO<sub>2</sub> and fire in promoting the spread of grasslands and savannas. *Global Change Biology* 9: 973-982.
- Bond WJ. 2008. What limits trees in C<sub>4</sub> grasslands and savannas? *Annual Review of Ecology, Evolution and Systematics* 39: 641-59.
- Brits J, van Rooyen MW, van Rooyen N. 2002. Ecological impact of large herbivores on the woody vegetation at selected watering points on the eastern basaltic soils in the Kruger National Park. *African Journal of Ecology* 40: 53-60.
- Cain ML, Damman H, Muir A. 1998. Seed dispersal and the Holocene migration of woodland herbs. *Ecological Monographs* 68: 325-347.
- Campbell ML, Clarke PJ. 2006. Seed dynamics of resprouting shrubs in grassy woodlands: seed rain, predators and seed loss constrain recruitment potential. *Austral Ecology* 31: 1016-1026.
- Clark JS, Macklin E, Wood L. 1998. Stages and spatial scales of recruitment limitation in southern Appalachian forests. *Ecological Monographs* 68: 213-235.
- Clark CJ, Poulsen JR, Levey DJ, Osenberg CW. 2007. Are plant populations seed limited? A critique and meta-analysis of seed addition experiments. *American Naturalist* 170: 129-142.

- Coates-Palgrave M. 2002. *Trees of southern Africa* (3<sup>rd</sup> ed.). Struik, Cape Town, South Africa.
- Coe M, Coe C. 1987. Large herbivores, *Acacia* trees and bruchid beetles. *South African Journal of Science* 83: 624-635.
- Cramer MD, Chimphango SBM, van Cauter A, Waldram MS, Bond WJ. 2007. Grass competition induces N<sub>2</sub> fixation in some species of African *Acacia*. *Journal of Ecology* 95: 1123-1133.
- Cumming DHM. 1982. The influence of large herbivores on savanna structure in Africa. In: Huntley BJ, Walker BH (eds), *Ecology of Tropical Savannas*. Springer, Berlin. pp 217-246.
- Davis MA, Wrage KJ, Reich PB. 1998. Competition between tree seedlings and herbaceous vegetation: support for a theory of resource supply and demand. *Journal of Ecology* 86: 652-661.
- El Aich A, Waterhouse A. 1999. Small ruminants in environmental conservation. *Small Ruminant Research* 34: 271-287.
- Eriksson O, Ehrlén J. 1992. Seed and microsite limitation of recruitment in plant populations. *Oecologia* 91: 360-364.
- Frost P, Medina E, Menaut JC, Solbrig O, Swift M, Walker B. 1986. Responses of savannas to stress and disturbances. *Biology International* 10: 1-8.
- Fuhlendorf SD. 1999. Ecological considerations for woody plant management. *Rangelands* 21: 12-15.

- Gardener CJ, McIvor JG, Jansen A. 1993. Passage of legume and grass seeds through the digestive tract of cattle and their survival in faeces. *Journal of Applied Ecology* 30: 63-74.
- Grellier S, Barot S, Janeau J, Ward D. 2012. Grass competition is more important than seed ingestion by livestock for *Acacia* recruitment in South Africa. *Plant Ecology* 213: 899-908.
- Grover HD, Musick HB. 1990. Shrubland encroachment in southern New Mexico, U.S.A.: an analysis of desertification processes in the American Southwest. *Climate Change* 17: 305-330.
- Hagos MG, Smit GN. 2005. Soil enrichment by *Acacia mellifera* subsp. *detinens* on nutrient-poor sandy soil in a semi-arid southern African savanna. *Journal of Arid Environments* 61: 47-59.
- Herrera CM, Jordano P, López-Soria L, Amat J. 1994. Recruitment of a mast-fruiting, bird dispersed tree: bridging frugivore activity and seedling establishment. *Ecological Monographs* 64: 315-344.
- Higgins SI, Bond WJ, Trollope WSW. 2000. Fire, resprouting and variability: a recipe for grass-tree coexistence in savanna. *Journal of Ecology* 88: 213-229.
- Hoffmann WA. 1996. The effects of fire and cover on seedling establishment in a neotropical savanna. *Journal of Ecology* 84: 383-393.

- House J, Archer S, Breshears DD, Scholes RJ and NCEAS Tree-Grass Interaction Participants. 2003. Conundrums in mixed woody-herbaceous plant systems. *Journal of Biogeography* 30: 1763-1777.
- Illius AW, O'Connor TG. 1999. On the relevance of non-equilibrium concept to arid and semi-arid grazing systems. *Ecological Applications* 9: 798-813.
- Jacobs N. 2000. Grasslands and thickets: bush encroachment and herding in the Kalahari thornveld. *Environment and History* 6: 289-316.
- Janzen DH. 1984. Dispersal of small seeds by big herbivores: foliage is the fruit. *American Naturalist* 123: 338-353.
- Jeltsch F, Milton SJ, Dean WRJ, van Rooyen N. 1996. Tree spacing and coexistence in semiarid savannas. *Journal of Ecology* 84: 583-595.
- Jeltsch F, Milton SJ, Dean WRJ, van Rooyen N, Moloney KA. 1998. Modelling the impact of small-scale heterogeneities on tree-grass coexistence in semi-arid savannas. *Journal of Ecology* 86: 780-794.
- Jeltsch F, Moloney KA, Milton SJ. 1999. Detecting process from snap-shot pattern: lessons from tree spacing in the southern Kalahari. *Oikos* 85: 451-467.
- Jeltsch F, Weber GE, Grimm V. 2000. Ecological buffering mechanisms in savannas: a unifying theory of long-term tree-grass coexistence. *Plant Ecology* 161: 161-171.

- Kgosikoma OE, Harvie BA, Mojeremane W. 2012. Bush encroachment in relation to rangeland management systems and environmental conditions in Kalahari ecosystem of Botswana. *African Journal of Agricultural Research* 7: 2312-2319.
- Kraaij T, Ward D. 2006. Effects of rain, nitrogen, fire and grazing on tree recruitment and early survival in bush-encroached savanna. *Plant Ecology* 186: 235 -246.
- Lacey JR, Wallander R, Olson-Rutz K. 1992. Recovery, germinability and viability of leafy spurge (*Euphorbia esula*) seeds ingested by sheep and goats. *Weed Technology* 6: 599-602.
- Lambers H, Chapin FS, Pons TL. 1998. *Plant physiological ecology*. Springer, New York, USA.
- Lamprey HF, Halevy G, Makacha S. 1974. Interactions between *Acacia*, bruchid beetles and large herbivores. *African Journal of Ecology* 12: 81-85.
- Lehmann CER, Archibald SA, Hoffmann WA, Bond WJ. 2011. Deciphering the distribution of the savanna biome. *New Phytologist* 19: 197-209.
- Materechera EK, Materechera SA. 2001. Breaking dormancy to improve germination in seeds of *Acacia erioloba*. *South African Journal of Plant and Soil* 18: 142-146.
- McNaughton SJ. 1992. The propagation of disturbances in savannas through food webs. *Journal of Vegetation Science* 3: 301-314.
- Miller MF, Coe M. 1993. Is it advantageous for *Acacia* seeds to be eaten by ungulates? *Oikos* 66: 364-368.

- Miller MF. 1994a. The interactions between vertebrates, bruchids and *Acacia* seeds. *Oecologia* 97: 265-270.
- Miller MF. 1994b. The fate of mature African *Acacia* pods and seeds during their passage from the tree to the soil. *Journal of Tropical Ecology* 10: 183-196.
- Miller MF. 1996. Dispersal of *Acacia* seeds by ungulates and ostriches in an African savanna. *Journal of Tropical Ecology* 12: 345-356.
- Milton SJ, Dean WRJ. 1995. South Africa's arid and semi-arid rangelands: why are they changing and can they be restored? *Environmental Monitoring and Assessment* 37: 245-264.
- Mlambo V, Smith T, Owen E, Mould FL, Sikosana JLN, Mueller-Harvey I. 2004. Tanniferous *Dichrostachys cinerea* fruits do not require detoxification for goat nutrition: *in sacco* and *in vivo* evaluations. *Livestock Production Science* 90: 135-144.
- Muller-Landau HC, Wright SJ, Calderón O, Hubbell SP, Foster RB. 2002. Assessing recruitment limitation: concepts, methods and case-studies from a tropical forest. In: Levey DJ, Silva WR, Galetti M (eds), *Seed dispersal and frugivory: ecology, evolution and conservation*. CAB International, Wallingford, UK. pp. 35-53.
- Nathan R, Muller-Landau H. 2000. Spatial patterns of seed dispersal, their determinants and consequences for recruitment. *Trends in Ecology and Evolution* 15: 278-285.



- Noy-Meir I. 1982. Stability of plant-herbivore models and possible application to savanna. In: Huntley BJ, Walker BH (eds), *Ecology of tropical savannas*. Springer, Berlin, Germany. pp 591-609.
- O'Connor TG. 1995. *Acacia karroo* invasion of grassland: environmental and biotic effects influencing seedling emergence and establishment. *Oecologia* 103: 214-223.
- O'Connor TG. 1996. Individual, population and community response of woody plants to browsing in African savannas. *Bulletin of the Grassland Society of southern Africa* 7, Supplement 1: 14-18.
- Or K, Ward D. 2003. Three-way interactions between *Acacia*, large mammalian herbivores and bruchid beetles - a review. *African Journal of Ecology* 41: 257-265.
- Or K, Ward D. 2004. The effects of seed quality and pipercolic and djenkolic acids on bruchid beetle infestation in water deficit-stressed *Acacia* trees. *Journal of Chemical Ecology* 30: 2291-2302.
- Rebertus AJ, Burns BR. 1997. The importance of gap processes in the development and maintenance of oak savannas and dry forests. *Journal of Ecology* 85: 635-645.
- Reid RS, Ellis JE. 1995. Impacts of pastoralists on woodlands in South Turkana, Kenya: livestock-mediated tree recruitment. *Ecological Applications* 5: 978-992.
- Richter CGF, Snyman HA, Smit GN. 2001. The influence of tree density on the grass layer of three semi-arid savanna types of southern Africa. *African Journal of Range and Forage Science* 18: 1-7.

- Rodriguez-Perez J, Wiegand K, Ward D. 2011. Interaction between ungulates and bruchid beetles and its effect on *Acacia* trees-modelling the cost and benefits of seed dispersal on plant demography. *Oecologia* 167: 97-105.
- Rohner C, Ward D. 1997. Chemical and mechanical defense against herbivory in two sympatric species of desert *Acacia*. *Journal of Vegetation Science* 8: 717-726.
- Rohner C, Ward D. 1999. Large mammalian herbivores and the conservation of arid *Acacia* stands in the Middle East. *Conservation Biology* 13: 1162-1171.
- Roques KG, O'Connor TG, Watkinson AR. 2001. Dynamics of shrub encroachment in an African savanna: relative influences of fire, herbivory, rainfall and density dependence. *Journal of Applied Ecology* 38: 268-280.
- Salazar A. 2010. *Seed dynamics and seedling establishment of woody species in the tropical savannas of Central Brazil (Cerrado)*. Unpubl. PhD thesis, University of Miami, Florida, USA.
- Sankaran M, Ratnam J, Hanan NP. 2004. Tree-grass co-existence in savannas revisited – insights from an examination of assumptions and mechanisms invoked in existing models. *Ecology Letters* 7: 480-490.
- Scholes RJ, Archer SR. 1997. Tree-grass interactions in savannas. *Annual Review of Ecology and Systematics* 28: 517-544.
- Scholes RJ. 2009. Syndromes of dryland degradation in southern Africa. *African Journal of Range and Forage Science* 26: 113-125.

- Schupp EW, Fuentes M. 1995. Spatial patterns of seed dispersal and the unification of plant population ecology. *Ecoscience* 2: 267-275.
- Skarpe C. 1986. Plant community structure in relation to grazing and environmental changes along a north-south transect in the western Kalahari. *Plant Ecology* 68: 3-18.
- Skarpe C. 1990. Structure of the woody vegetation in disturbed and undisturbed arid savanna. *Plant Ecology* 87: 11-18.
- Skarpe C. 1991. Spatial patterns and dynamics of woody vegetation in an arid savanna. *Journal of Vegetation Science* 2: 565-572.
- Skarpe C. 1992. Dynamics of savanna ecosystems. *Journal of Vegetation Science* 3: 293-300.
- Smit GN, Richter CGF, Aucamp AJ. 1999. Bush encroachment: an approach to understanding and managing the problem. In: Tainton, NM (ed.), *Veld management in South Africa*. University of Natal Press, Pietermaritzburg, South Africa. pp 246-260.
- Smit GN. 2004. An approach to tree thinning to structure southern African savannas for long term restoration from bush encroachment. *Journal of Environmental Management* 71: 179-191.
- Stuart-Hill GC, Tainton NM. 1989. The competitive interaction between *Acacia karroo* and the herbaceous layer and how this is influenced by defoliation. *Journal of Applied Ecology* 26: 285-298.
- Sullivan S, Rohde R. 2002. On non-equilibrium in arid and semi-arid grazing systems. *Journal of Biogeography* 29: 1595-1618.

- Tews J, Moloney K, Jeltsch F. 2004. Modeling seed dispersal in a variable environment: a case study of the fleshy-fruited savanna shrub *Grewia flava*. *Ecological Modelling* 175: 65-76.
- Trollope WSW. 1980. Controlling bush encroachment with fire in the savanna areas of South Africa. *Proceedings of the Grassland Society of southern Africa* 15: 173-177.
- Trollope WSW. 1982. Ecological effects of fire in South African savannas. In: Huntley BJ, Walker BH (eds), *Ecology of tropical savannas*. Springer, Heidelberg, Germany. pp 292-306.
- Turnbull LA, Crawley MJ, Rees M. 2000. Are plant populations seed-limited? A review of seed sowing experiments. *Oikos* 88: 225-238.
- van Auken OW, Bush JK. 1989. *Prosopis glandulosa* growth: influence of nutrients and simulated grazing of *Bouteloua curtipendula*. *Ecology* 70: 512-516.
- van Auken OW. 2009. Causes and consequences of woody plant encroachment into western North American grassland. *Journal of Environmental Management* 90: 2931-2942.
- van Langevelde F, van de Vijver C, Kumar L, van de Koppel J, de Ridder N, van Andel J, Skidmore AK, Hearne JW, Stroosnijder L, Bond WJ, Prins HHT, Rietkerk M. 2003. Effects of fire and herbivory on the stability of savanna ecosystems. *Ecology* 84: 337-350.
- van Vegten JA. 1983. Thornbush invasion in a savanna ecosystem in eastern Botswana. *Plant Ecology* 56: 3-7.

- Walker BH, Ludwig D, Holling CS, Peterman RM. 1981. Stability of semi-arid savanna grazing systems. *Journal of Ecology* 69: 473-498.
- Walker BH, Noy-Meir I. 1982. Aspects of the stability and resilience of savanna ecosystem. In: Huntley BJ, Walker BH (eds). *Ecology of tropical savannas*. Springer, Berlin, Germany. p. 556-590.
- Walter H. 1939. Grassland, savanne und busch der arideren Teile Afrikas in ihrer ökologischen Bedingtheit. *Jahrbucher für Wissenschaftliche Botanik* 87: 750-860.
- Ward D. 2005. Do we understand the causes of bush encroachment in African savannas? *African Journal of Range and Forage Science* 22: 101-106.
- Ward D. 2010. A resource ratio model of the effects of changes in CO<sub>2</sub> on woody plant invasion. *Plant Ecology* 209: 147-152.
- Ward D, Esler KJ. 2010. What are the effects of substrate and grass removal on recruitment of *Acacia mellifera* seedlings in a semi-arid environment? *Plant Ecology* 212: 245-250.
- Ward D, Musli I, Or K, Gbenro T, Skutelsky O. 2010. Bruchid seed infestation and development time in three host species of *Acacia*. *Zoology in the Middle East* 51: 95-103.
- Ward D, Wiegand, K, Getzin S. 2013. Walter's two-layer hypothesis revisited: back to the roots! *Oecologia* 172: 617-630.
- Weitbrecht K, Müller K, Leubner-Metzger G. 2011. First off the mark: early seed germination. *Journal of Experimental Botany* 62: 3289-3309.

- Westoby M, Leishman M, Lord J. 1996. Comparative ecology of seed size and dispersal. *Philosophical Transactions of the Royal Society of London, Series B* 351: 1309-1318.
- Wiegand K, Ward D, Saltz D. 2005. Multi-scale patterns and bush encroachment in an arid savanna with a shallow soil layer. *Journal of Vegetation Science* 16: 311-320.
- Wiegand K, Saltz D, Ward D. 2006. A patch-dynamics approach to savanna dynamics and woody plant encroachment – insights from an arid savanna. *Perspectives in Plant Ecology, Evolution and Systematics* 7: 229-242.
- Wigley BJ, Bond WJ, Hoffman MT. 2010. Thicket expansion in a South African savanna under divergent land use: local vs. global drivers? *Global Change Biology* 16: 964-976.
- Zammit C, Westoby M. 1987. Seedling recruitment strategies in obligate-seeding and resprouting *Banksia* shrubs. *Ecology* 68: 1984-1992.

## 2. Chapter 2

### **Recovery and germination of *Dichrostachys cinerea* seeds fed to goats (*Capra hircus*)**

Julius T. Tjelele,<sup>1</sup> Luthando E. Dziba,<sup>2</sup> and Hosea T. Pule<sup>3</sup>

Authors are Researcher, <sup>1</sup> Agricultural Research Council, Animal Production Institute, Private Bag X 02, Irene, 0062, South Africa; Principal Researcher,<sup>2</sup> CSIR: Natural Resources and the Environment, P O Box 395, Pretoria 0001, South Africa; and Researcher,<sup>3</sup> Agricultural Research Council, Animal Production Institute, Private Bag X 02, Irene, 0062, South Africa

Corresponding Author: Julius T. Tjelele, Agricultural Research Council, Animal Production Institute, Private Bag X2, Irene, 0062, South Africa.

Email: jtjelele@arc.agric.za

**Published in *Rangeland Ecology and Management* as: Tjelele, J.T. , Dziba, L.E. & Pule, H.T. (2012) Recovery and Germination of *Dichrostachys cinerea* Seeds Fed to Goats (*Capra hircus*). *Rangeland Ecology & Management* 65: 105-108.**

**doi: <http://dx.doi.org/10.2111/REM-D-09-00161.1>**

## 2.1. Abstract

Goats can act as dispersal agents by consuming seed pods of woody plants and dispersing the seeds in feces. Concerns that goats might thereby promote encroachment by woody plant species such as *Dichrostachys cinerea* (sickle bush) have not been addressed. The objective of this study was to determine the recovery rate and germination potential of *D. cinerea* seeds that pass through the digestive tract of goats. We hypothesized that 1) *D. cinerea* seeds will remain intact and viable after passage through the digestive tract of goats; and 2) *D. cinerea* seeds will be scarified by such passage, resulting in improved germination percentages. The first trial measured the recovery rate of 1500 *D. cinerea* seeds that were consumed by indigenous goats, either voluntarily, after mixing them with feed pellets (mixed), or by force-feeding (gavaged). Seed recovery for the gavaged treatment (32.7%) was significantly higher than for the mixed treatment (9.9%;  $P < 0.001$ ). The second trial determined germination percentages of *D. cinerea* seeds recovered from the feces of animals in the two treatments of the first trial, as well as scarified and control (untreated) seeds. The germination percentage of mechanically scarified seeds (53.0%) was significantly higher than that of seeds that passed through the digestive system in the mixed (35.5%) or gavaged (31.2%) treatments, or were untreated (19.0%;  $P < 0.001$ ). Seeds that passed through the digestive tract (mixed and gavaged treatments) had a significantly higher germination percentage than untreated seeds ( $P < 0.001$ ). A non-negligible proportion of *D. cinerea* seeds remained intact after ingestive chewing and passage through the digestive system, and their germination percentage was even elevated. This suggests that goats have a potential to facilitate woody plant encroachment through dispersal of viable and scarified seeds.



**Key words:** gavaging, sickle bush, seed germination, rumen digestion, scarification, seed recovery, seed survival, woody plant encroachment

## 2.2. Introduction

Woody plant encroachment has been reported throughout the savannas of southern Africa (van Vegten 1983; Skarpe 1986; Smit 2004). It is a phenomenon whereby trees and shrubs invade grassland and/or increase in density in an already wooded area, resulting in lower yields of herbaceous plants and a reduction in the carrying capacity of rangelands (Richter et al. 2001; Ward 2005). It is not only the extent of woody plant encroachment but also the rate at which it occurs that is a major concern (Kraaij and Ward 2006). The question of what causes woody plant encroachment still remains unanswered (Ward 2005; Wiegand et al. 2006) and this inhibits development of effective management.

Herbivores browsing certain woody plants may consume fruits and/or seed pods and disperse seeds through their dung (Janzen 1984; Fuhlendorf 1999; Tews et al. 2004). Seeds dispersed by animals may contribute to an increase in bush encroachment alone, or in combination with other factors such as global climate change, heavy grazing and reduced fire frequency (O'Connor 1996; Schupp et al. 1997; Bond et al. 2003; Ward 2005).

In savannas, fire and goats have been used as tools to manage woody plant encroachment (Trollope 1980; El Aich and Waterhouse 1999). Yet, the role of goats in the dispersal of *Dichrostachys cinerea* (sickle bush) seeds and the germination percentages of seeds dispersed by goats remains unknown and a major concern for land users.

*D. cinerea* is a thorny tree that is one of the common encroachers of South African savannas. The crown-like umbrella structure and its relatively impenetrable canopy constrain browsing by large mammalian herbivores. *D. cinerea* suppresses the herbaceous layer and reduces rangeland carrying capacity and livestock production (Richter et al. 2001).

The main objective of this study was to determine the recovery and germination of *D. cinerea* seeds that passed through the digestive tract of goats. We hypothesized that 1) *D. cinerea* seeds will remain intact and viable after passage through the digestive tract of goats; and 2) passage of *D. cinerea* seeds through the digestive tract of goats will facilitate scarification of the seed-coat and improve germination percentages.

### **2.3. Materials and methods**

#### **2.3.1 Seed collection**

Dry mature pods of *D. cinerea* were collected from trees and under trees at Kwa-Mhlanga, Mpumalanga province (28°30'E, 25°15'S), approximately 95 km north of Pretoria, where this species occurs abundantly. Five different *D. cinerea* trees were used to collect pods. Undamaged seeds were removed from the pods, immersed in water, and floating seeds were discarded. Cleaned seeds were stored in paper bags at room temperature (20°-23°C) (Lacey et al. 1992). Sorting on the basis of density was done before storage to exclude seeds that were infected or not fully ripened. Three replicates of 1 000 seeds were weighed to obtain an average seed weight and the lengths and widths of 100 seeds were measured using a caliper. Mean seed weight was 27.56 mg (SE±0.23), while the mean length and width were 5.06 mm (SE±0.04) and 2.22 mm (SE±0.02), respectively.

### **2.3.2 Animals and feeding**

A total of twenty female indigenous goats (South African veld goats) were used for the study, with an average weight of 20.4 kg (SE±0.71). The study was conducted at the Agricultural Research Council (ARC) Irene Experimental Farm in Pretoria, Gauteng province. Goats were placed individually in (2×1)-m pens and were fed ram-lamb and ewe pellets (Epol (Pty) Ltd, Pretoria) *ad libitum* for 24 days prior to the experiment to allow them to acclimate to experimental conditions and clean any possible seeds from the digestive tract, as well as during the experiment. The pellets contained 13% protein, 14% crude fibre, 1.5% calcium and 0.2% phosphorus on a dry matter basis, and were 14.80 mm (SE±0.75) long and 4.89 mm (SE±0.03) wide. Fresh drinking water and salt blocks were also provided *ad libitum*.

### **2.3.3 Seed recovery trial**

Seed recovery was investigated using two methods of feeding goats – voluntary feeding of seeds mixed with diet and force-feeding – in order to separate the effects of ingestive chewing from those of digestion (including rumination chewing). In the first method, 10 goats were fed 1 500 *D. cinerea* seeds mixed with a basal diet of ram-lamb and ewe pellets (mixed). Goats were allowed to consume seeds within 24 h after which the refusals were collected and unconsumed seeds were sorted and counted. In the second method, 10 goats were force-fed (gavaged) with 1 500 *D. cinerea* seeds. Gavaging seeds directly into the stomach ensured that a known quantity of seeds is ingested (Barrow and Havstad 1992). The amount of seeds fed to goats in this experiment was based on the level used by Lacey et al. (1992).

Feces collection in both treatments commenced 24 h after feeding and continued for eleven days, by which time no seeds were found in the feces. All feces excreted by each goat were

collected twice daily from the concrete floor. Feces were immersed in cold water until soft and then washed with tap water through a wire strainer until the water was clear. A light cabinet was used to separate seeds from fecal remains. Undamaged (intact) seeds that were recovered from the feces for each goat for that day were counted and stored in brown paper bags in a cool dry place until the end of the trial.

#### ***2.3.4 Germination trial***

Germination potential was determined for seeds subjected to four treatments: 1) mixed seed, 2) gavigated seed, 3) mechanically scarified seeds and 4) control (untreated seeds). Each treatment consisted of four replicates of 50 seeds per replicate. Seeds for the mixed and gavigated treatments were drawn randomly from the pooled collection of seeds that were recovered from the feces of goats that had been allocated to these treatments in the seed recovery trial. The scarified seed treatment used seeds that had been mechanically scarified using sandpaper (Baes et al. 2002).

Germination tests were conducted at the ARC Roodeplaat Farm Forage Genebank according to the standards of the International Seed Testing Association (ISTA) (1985). The germination tests used 12 mm square plastic dishes containing one disc of germination paper and 5 ml distilled water, and were run in a germination chamber kept at a temperature of 20-30°C with 16 h dark period and 8 h light period. Each plastic dish contained 50 seeds. The germination trial was monitored for a period of up to 16 d and all germinated seeds were recorded and removed. All seeds that did not germinate were counted and the percentage germination was calculated as the number of seeds germinated divided by the total number of seeds placed in a petri dish  $\times 100$  (Armke and Scott 1999).

### 2.3.5 Statistical analysis

In the seed recovery trial, the effect of two seed feeding methods (gavaged seed and mixed seed) on seed recovery was analyzed using t-test. The total seed recovery from goats was the dependent variable. Each goat was considered an experimental unit ( $n=20$ ). The data were log-transformed for normal distribution. In the germination trial, analysis of variance was used to evaluate significant differences in germination percentages among the four seed treatments (gavaged seed, mixed seed, mechanically scarified and control). The total germination percentage was the dependent variable. Differences between means were considered significant at  $\alpha = 5\%$  level. SAS software (2002) was used for data analysis.

## 2.4. Results and Discussion

Goats consumed fewer seeds (1,298 (SE  $\pm$  9.58) seeds/goat) when allowed to voluntarily feed on seeds mixed in a ration than when the seeds were gavaged (1,500 seeds/goat). Seed recovery for the gavaged seed treatment was significantly higher than for the mixed feed treatment ( $P < 0.001$ ) (Figure 1). A sharp increase in seed recovery for gavaged compared to mixed seed treatments may be due to the rate at which seeds entered the digestive system: as a single massive pulse for gavaged seeds versus a slow trickle for the mixed seed treatment. The morphology of *D. cinerea* seeds (small hard seeds) may explain the generally rapid initial rate of passage for both treatments (Whitacre and Call 2006). Seeds that are  $<2.5$  mm in width usually have higher recovery during the first few days after consumption than later in the period because they more readily separate from fibrous digesta in the rumen (Gardener et al. 1993; Whitacre and Call 2006). Although seed recovery from the two seed feeding methods followed the same trend (a larger percentage being recovered earlier), cumulative percentage seed recovery for the

gavaged seed treatment was higher (32.7%), compared to 9.9% for the mixed seed treatment (Figure 1), suggesting some damaging effects of ingestive chewing. This study, therefore, partially supported the hypothesis that *D. cinerea* seeds pass through the rumen of goats intact and viable.

The germination percentage of mechanically-scarified seeds was higher (53.0%) compared to gavaged (31.2%), mixed (35.5%) and control (19.0%) seed treatments ( $P < 0.001$ ). Rumen-treated seeds (gavaged and mixed seed) had a higher germination percentage than control seeds ( $P < 0.001$ ). The hypothesis that *D. cinerea* seeds that pass through the digestive tract of goats will have higher seed germination percentages compared to untreated seeds was supported. The hard coat of *D. cinerea* seeds together with the faster passage rates may explain the viability of the recovered seeds and the improved germination due to adequate scarification without substantial damage to some of the seeds (Brown and Archer 1987; Miller 1995).

The germination percentages of mechanically-scarified and the control treatment increased rapidly during the first 10 days compared to days 11- 16 (Figure 2). The seed germination percentages obtained from gavaged and mixed seed treatments suggest that passage of seeds through the digestive tract of goats improves seed germination compared to seed germinated from untreated (control) seeds. However, many factors influence germination percentages, including the quality of diet, animal species, plant species, and seed characteristics (Simao Neto et al. 1987; Whitacre and Call 2006). All these factors need to be investigated collectively to determine the extent to which herbivores contribute to spreading of viable seeds via the dung and how this may subsequently lead to woody plant encroachment.

## **2.5. Management Implications**

Results from the current study showed that although there was a lower percentage of seeds that passed intact through the digestive tract of goats, those seeds remained viable and had substantial germination potential. Although the relative viability of seeds that passed through the rumen was lower than mechanically scarified, it was nearly double compared to that of untreated seeds. The small relative loss in viability of ingested seeds is a good tradeoff for the likelihood of these seeds being dispersed by animals away from the tree. We conclude that goats have a potential to facilitate woody plant encroachment through dispersal of viable and partially scarified seeds that have potential to germinate. Understanding other factors that influence seed viability and germination following ingestion by herbivores and the evolutionary seed dispersal strategies of encroaching tree species may aid attempts to manage woody plant encroachment.

## **2.6. Acknowledgements**

The Agricultural Research Council (ARC) and the Gauteng Department of Agriculture and Rural Development provided financial support for this project. The study was approved by ARC Animal Ethics Committee. We are grateful to ARC staff for technical support. We also thank David Ward, Tsedal Ghebremariam and two anonymous reviewers for their comments.

## 2.7. References

- Armke, F.W., and C.B. Scott. 1999. Using cattle to disperse seeds for winter forage plants. *Texas Journal of Agriculture and Natural Resources* 12:28-38.
- Baes, P.O., M.L. de Viana, and S. Suhring. 2002. Germination in *Prosopis ferox* seeds: effects of mechanical, chemical and biological scarificators. *Journal of Arid Environments* 50:185-189.
- Barrow, J.R., and K.M. Havstad. 1992. Recovery and germination of gelatin-encapsulated seeds fed to cattle. *Journal of Arid Environments* 22:395-399.
- Bond, W. J., G.F. Midgley, and W.I. Woodward. 2003. The importance of low atmospheric CO<sub>2</sub> and fire in promoting the spread of grasslands and savannas. *Global Change Biology* 9: 973-982.
- Brown, J.R., and S. Archer. 1987. Woody plant seed dispersal and gap formation in a North American subtropical savanna woodland: the role of domestic herbivores. *Plant Ecology* 73:73-80.
- El Aich, A., and A. Waterhouse. 1999. Small ruminants in environmental conservation. *Small Ruminant Research* 34:271-287.
- Fuhlendorf, S.D. 1999. Ecological considerations for woody plant management. *Rangelands* 21:12-15.
- Gardener, C.J., J.G. Mclvor, and A. Jansen. 1993. Passage of legume and grass seeds through the digestive tract of cattle and their survival in faeces. *Journal of Applied Ecology* 30:63-74.



- International Seed Testing Association. 1985. International rules for seed testing. *Seed Science and Technology* 13:299-355.
- Janzen, D.H. 1984. Dispersal of small seeds by big herbivores: foliage is the fruit. *American Naturalist* 123:338-353.
- Kraaij, T., and D.H. Ward. 2006. Effects of rain, nitrogen, fire and grazing on tree recruitment and early survival in bush-encroached savanna, South Africa. *Plant Ecology* 186:235-246.
- Lacey, J.R., R. Wallander, and K. Olson-Rutz. 1992. Recovery, germinability, and viability of leafy spurge (*Euphorbia esula*) seeds ingested by sheep and goats. *Weed Technology* 6:599-602.
- Miller, M.F. 1995. *Acacia* seed survival, seed germination and seedling growth following pod consumption by large herbivores and seed chewing rodents. *African Journal of Ecology* 33:194-210.
- O'Connor, T.G. 1996. Individual, population and community response of woody plants to browsing in African savannas. *Bulletin of the Grassland Society of Southern Africa, Supplement* 7:14-18.
- Richter, C.G.F., H.A. Snyman, and G.N. Smit. 2001. The influence of tree density on the grass layer of three semi-arid savanna types of southern Africa. *African Journal of Range and Forage Science* 18:103–109.
- SAS Institute. 2002. Version 9.2. Cary, NC, USA: SAS Institute, Inc.

- Schupp, E.W., J.M. Gomes, J.E. Jimenez, and M. Fuentes. 1997. Dispersal of *Juniperus occidentalis* (Western Juniper) seeds by frugivorous mammals on Juniper Mountain, southeastern Oregon. *Great Basin Naturalist* 57:74-78.
- Simao Neto, M., R.M. Jones, and D. Ratcliff. 1987. Recovery of pasture seed ingested by ruminants. 1. Seed of six tropical pasture species fed to cattle, sheep and goats. *Australian Journal of Experimental Agriculture* 27:239-246.
- Skarpe, C. 1986. Plant community structure in relation to grazing and environmental changes along a north-south transect in the western Kalahari. *Vegetatio* 68:3-18.
- Smit, G.N. 2004. An approach to tree thinning to structure southern African savannas for long-term restoration from bush encroachment. *Journal of Environmental Management* 71:179-191.
- Tews, J., K. Moloney, and F. Jeltsch. 2004. Modeling seed dispersal in a variable environment: a case study of the fleshy-fruited savanna shrub *Grewia flava*. *Ecological Modelling* 175:65-76.
- Trollope, W.S.W. 1980. Controlling bush encroachment with fire in the savanna areas of South Africa. *Proceedings of the Grassland Society of Southern Africa* 15:173-177.
- van Vegten, J.A. 1983. Thornbush invasion in a savanna ecosystem in eastern Botswana. *Vegetatio* 56:3-7.
- Ward, D. 2005. Do we understand the causes of bush encroachment in African savannas? *African Journal of Range and Forage Science* 22:101-105.

Wiegand, K., D. Saltz, and D. Ward. 2006. A patch-dynamics approach to savanna dynamics and woody plant encroachment – Insights from an arid savanna. *Perspectives in Plant Ecology, Evolution and Systematics* 7:229-242.

Whitacre, M.K., and C.A Call. 2006. Recovery and germinability of native seed fed to cattle. *Western North American Naturalist* 66:121-128.

## 2.8. Figure Legend

Figure 1. Cumulative recovery (as % of seed consumed) of *D. cinerea* seeds from goats fed using two methods (mixed seed and gavaged seed). Error bars represent standard errors (SE).

Figure 2. Cumulative seed germination percentage among four treatments: mixed seed, gavaged seed, mechanically scarified seed, and control (untreated) seeds over three periods (days 0-4, 5-10 and 11-16). Error bars represent standard errors (SE).

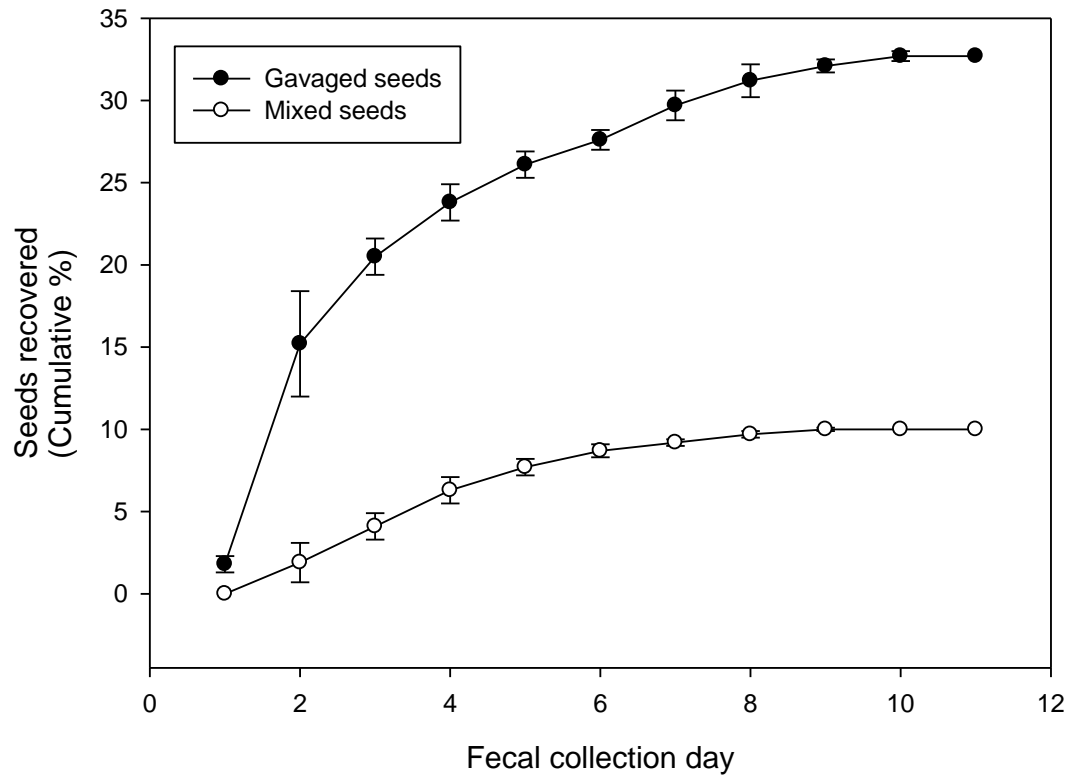


Figure 1

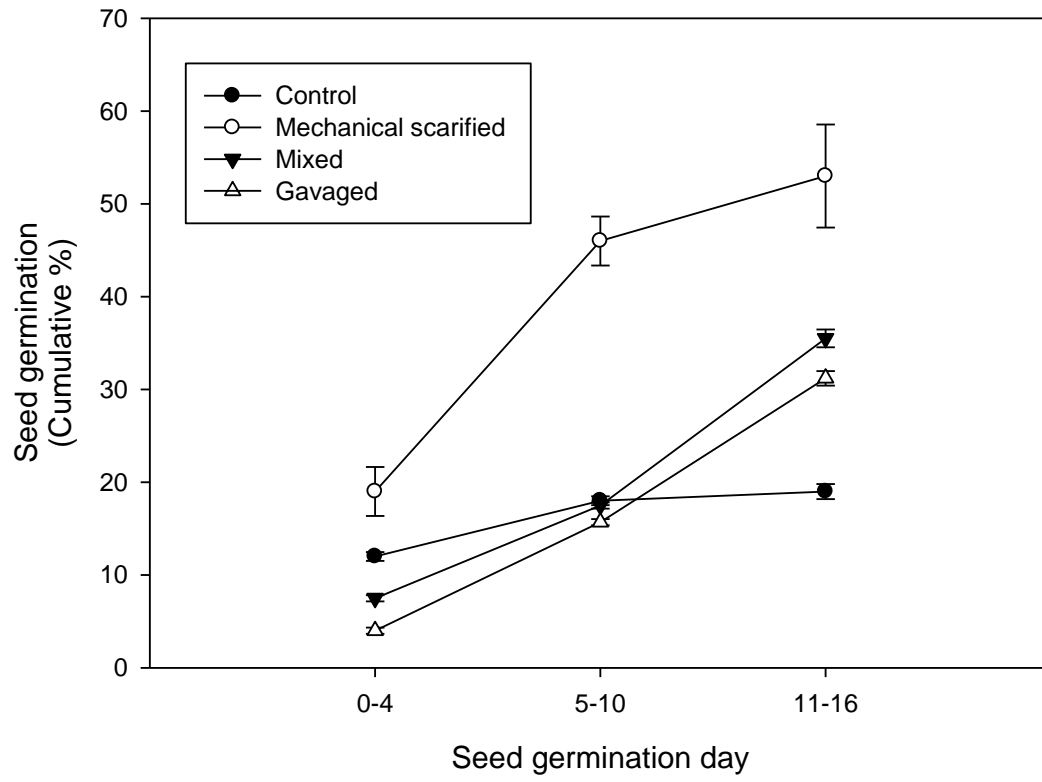


Figure 2

## Chapter 3

### Effects of diet quality on germination of *Dichrostachys cinerea* and *Acacia nilotica* seeds fed to ruminants

Julius Tjelele<sup>1,2</sup>, David Ward<sup>2</sup>, and Luthando Dziba<sup>3</sup>

Authors are Researcher, <sup>1</sup>Agricultural Research Council, Animal Production Institute, Irene, 0062, South Africa; Professor of Plant Ecology, <sup>2</sup>College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Scottsville 3209, South Africa; Principal Researcher, <sup>3</sup>CSIR: Natural Resources and the Environment, Pretoria 0001, South Africa

Research was funded by the Gauteng Department of Agriculture and Rural Development (GDARD), National Research Foundation (NRF) and the International Foundation for Science (IFS).

Correspondence author: Julius Tjelele, Agricultural Research Council, Animal Production Institute, Private Bag X2, Irene, 0062, South Africa. Email: [jtjelele@arc.agric.za](mailto:jtjelele@arc.agric.za)

Submitted for publication to *Rangeland Ecology and Management*

### 3.1. Abstract

The pods of many woody plant species form an important part of the diet of livestock during the dry season due to their high nutritive value. However, the dispersal of seeds that remain intact and can potentially germinate after excretion is of particular concern when animals consume seeds of encroaching or invasive woody plants. The objectives of this study were to determine the effect of animal species in two experiments (experiment 1: goats, sheep; experiment 2: goats, cattle), diet quality (*Medicago sativa* hay, *Digitaria eriantha* hay) and seed characteristics (size, hardness) on the effectiveness of animal seed dispersal and germination of *Dichrostachys cinerea* and *Acacia nilotica* seeds. Owing to a limitation on the availability of seeds, the two experiments (goats, sheep and goats, cattle) were done separately at different times. Each animal in experiment one and two received 1000 *A. nilotica* seeds and 1000 *D. cinerea* seeds mixed with either a low-quality diet (*D. eriantha* hay) or a high-quality diet (*M. sativa* hay). In the first experiment, a significant interaction effect of animal species (goats, sheep), diet (high-quality hay, low-quality hay) and seed species (*A. nilotica* seeds, *D. cinerea* seeds) was found on percentage germination ( $P < 0.0001$ ). There was also a higher percentage seed recovery ( $P < 0.009$ ) when animals were offered high-quality hay ( $47.4\% \pm 4.65$ ) compared to low-quality hay ( $30.2\% \pm 3.24$ ). In goats fed *D. eriantha* hay, *A. nilotica* seed germination ( $9.38\% \pm 3.66$ ) was higher ( $P < 0.05$ ) than goats fed *D. eriantha* hay and *D. cinerea* seeds ( $6.78\% \pm 1.13$ ). A greater germination percentage was observed in goats fed *M. sativa* hay with *D. cinerea* seeds ( $6.71\% \pm 1.53$ ) than goats fed *M. sativa* hay with *A. nilotica* seeds ( $2.50\% \pm 0.97$ ) ( $P < 0.05$ ). In the second experiment, animal species had a positive impact, both on seed recovery ( $P < 0.0325$ ; goats  $32.0\% \pm 6.44$ ; cattle  $50.3\% \pm 4.27$ ) and germination percentage ( $P < 0.055$ ; goats  $14.1\% \pm$



1.48; cattle  $9.3\% \pm 0.94$ ; control: *D. cinerea*  $0.64 \pm 0.06$ ; *A. nilotica*  $0.59 \pm 0.07$ ). The animal species main effect in experiment one and two was most important for seed recovery and germination. However, diet (*M. sativa* hay and *D. eriantha* hay) and seed species (*D. cinerea*, *A. nilotica* seeds) also had important effects on germination of seeds retrieved from experiment one (goats, sheep). Thus, all three of these factors play a major role in recovery of viable and scarified seeds.

**Key words:** associated diet quality, dispersal, germination percentage, seed characteristics, seed viability, woody plant encroachment

### 3.2. Introduction

Seed dispersal by livestock involves a number of phases from seed ingestion to seedling establishment and survival (Gardener et al. 1993). Many seeds may be destroyed during passage through the digestive tract of herbivores while others are defecated undamaged in the feces (Rohner and Ward 1999; Or and Ward 2003). This may frequently be related to seed hardness. Consumption of hard seeds by livestock may facilitate seed germination by scarifying the seed coat and thereby increasing water uptake during passage through the digestive system (Hoffman et al. 1989; Miller 1995; Tjelele et al. 2012). Several factors such as animal species, body size and associated diet quality (e.g. *Medicago sativa* hay and *Digitaria eriantha* hay) play a significant role in seed recovery, seed germination or seed viability (Janzen 1984; Rohner and Ward 1999; Pakeman et al. 2002; Or and Ward 2003; Myers et al. 2004).

The survival or scarification of seeds during passage through the digestive tract of animals has vital implications for the population dynamics of plant species (Gardener et al. 1993; Nathan and Muller-Landau 2000). If seeds survive the digestive system they may subsequently be dispersed. However, there is usually a positive correlation between body size and percentage germination, which depends among other issues such as associated diet quality (Jones and Simao Neto 1987) and hardness of the seed coat (Gardener et al. 1993; Whitacre and Call 2006). Thus, seed recovery and germination may be correlated with diet quality, animal species, body size and seed species (Simao Neto et al. 1987; Shayo and Uden 1989; Whitacre and Call 2006).

Associated feed of high digestibility, high crude protein and low fiber passes more quickly through the digestive tract of animals (Robbins 1993; van Soest 1994), presumably carrying more seeds with less feces (Simao Neto and Jones 1987). Faster passage rate may result in low

scarification and low germination percentages (Brown and Archer 1987; Or and Ward 2003; Bodmer and Ward 2006).

Goats, sheep and cattle are possible seed dispersal agents affecting germination of *Dichrostachys cinerea* and *Acacia nilotica* seeds in African savannas. Goats are mixed feeders that are flexible in their dietary choices whereas sheep and cattle are mainly grazers, preferring to eat herbaceous material. However, in situations where there are many bushes available, sheep and cattle may also be browsers (El Aich and Waterhouse 1999). Large-bodied animals such as cattle do not chew seeds as completely as small ruminants, e.g. goats and sheep, which may result in high seed recovery from cattle with less damage (Simao Neto et al. 1987; Shayo and Uden 1989). Another consequence of large body size is greater seed retention than in sheep and goats, especially when offered diets with lower digestibility (Bodmer and Ward 2006). Bodmer and Ward (2006) found that there is a positive correlation between germination rates in *Acacia* seeds and size of the herbivore that ingests the seeds. This correlation is most likely due to allometric scaling of digestion time (passage rate) to herbivore body mass (Robbins 1993), resulting in greater germination and viability of seeds that have passed through the digestive tract of large herbivores. Thus seed size, seed hardness, associated diet quality and body size can influence retention times in the digestive tract and subsequently influence seed recovery, scarification and seed germination success (Simao Neto et al. 1987; Gardener et al. 1993; Or and Ward 2003; Bodmer and Ward 2006).

The objectives of this study were to determine how seed characteristics, diet quality and animal species influence seed recovery and germination of *D. cinerea* and *A. nilotica* to better understand the influence of seed dispersal by animals. Both *D. cinerea* and *A. nilotica* are known to be encroaching tree species, so factors that exacerbate their dispersal of viable and germinable

seeds are likely to be of major concern to ranchers attempting to limit woody plant encroachment. We hypothesized that 1) animals consuming a low-quality (*Digitaria eriantha* hay) diet will have longer seed retention time than when consuming a high-quality (*Medicago sativa* hay) diet, resulting in lower seed recovery and lower germination due to seed damage, 2) seeds fed to large-bodied animals (cattle) will result in high germination relative to small ruminants (goats, sheep) because the seeds remain in the gut for longer, enhancing scarification, and 3) *D. cinerea* seeds are more likely to escape mastication and rumination and should also germinate better than *A. nilotica* seeds because *D. cinerea* seeds are smaller.

### **3.3. Materials and methods**

#### ***3.3.1 Seed Collection***

Dry mature pods of *D. cinerea* and *A. nilotica* were collected from under trees in KwaMhlanga, Mpumalanga province (lat 28°30'E, long 25°15'S) and the Agricultural Research Council Farm, Gauteng province (lat 28°19'E, long 25°35'S), respectively. Undamaged seeds were removed by hand from pods. The seeds were immersed in fresh water and any floating seeds were discarded because they were either unripe or damaged by bruchid beetles (see Or and Ward 2003). The tetrazolium viability tests (International Seed Testing Association 2012) of *D. cinerea* and *A. nilotica* seeds prior to feeding them to animals indicated that they had 95 and 91% viability, respectively. The length and width of 100 *D. cinerea* seeds and 100 *A. nilotica* seeds were measured using vernier calipers. The densities for *A. nilotica* and *D. cinerea* seeds were measured using a Micro-focus X-ray Computed Tomography scanner. Seed characteristics (size, shape and hardness) may affect seed recovery, scarification and germination (Whitacre and Call 2006).

### **3.3.2 Animal Feeding and Sample Measurement**

Experimental data were subjected to a 2×2×2 factorial analysis of variance with the following factors: animal species (goats and sheep (experiment one)), goats and cattle (experiment two)), diets (low-quality hay and high-quality hay) and seed species (*D. cinerea* and *A. nilotica*). Due to logistical constraints on the availability of seeds, experiments one and two were done in February 2011 and 2012, respectively). We replicated the goat treatment to ensure comparability of results.

The studies were done at the Agricultural Research Council's, Irene Farm, Pretoria, South Africa. All animals were kept in the metabolic cages for 5 d to acclimate them to the experimental conditions and for 11 d during the seed recovery study. In both experiments, each animal was fed the experimental diet and seeds, individually in metabolic cages. All animals were fed ground *Medicago sativa* and *Digitaria eriantha* hay, and water *ad libitum* throughout the experiment (i.e. during acclimatization and seed recovery). Each animal received 1000 *A. nilotica* and 1000 *D. cinerea* seeds once at the beginning of the experiment mixed with the feed in the feeding trough.

Each day during the 11 d of seed recovery, a random grab sample of each feed was taken and bulked in a sealed bag pending the analysis. For each feed, the bulked sample was analyzed for crude protein using Kjeldahl block digestion method (Association of Official Analytical Chemist 2000), neutral detergent fibre using tector fibertec system (Van Soest, Robertson and Lewis 1991). *In vitro* digestibility of organic matter was done using the method based on Tilley and Terry (1963) as modified by Engels and Van der Merwe (1967).

### **3.3.3 Experiment one (goats and sheep)**

Twenty female indigenous goats (South African veld goats) and 20 female Dorper sheep were used in this study, with mean weights of  $22 \text{ kg} \pm 0.9$  and  $23 \text{ kg} \pm 0.9$ , respectively. The 20 animals of each species were divided into two groups of 10 each then each group of 10 was further divided into two groups of five per group and each animal was fed high-quality (*Medicago sativa*) hay (23% CP, 44% NDF and 70% *in vitro* digestibility organic matter (IVDOM)) mixed with either *D. cinerea* or *A. nilotica* seeds. The other group of 10 animals was divided similarly and animal of this group was fed low-quality (*Digitaria eriantha*) hay (3% CP, 72% NDF and 62% IVDOM) mixed with either *D. cinerea* (five animals) or *A. nilotica* seeds (five animals).

### **3.3.4 Experiment two (goats and cattle)**

Twenty Bonsmara cows and 20 female indigenous goats were used, with mean weights of  $215 \text{ kg} \pm 4.8$  and  $24 \text{ kg} \pm 0.9$ , respectively. The 20 animals of each species were divided into two groups of 10 each then each group of 10 was further divided into two groups of five per group and each animal was fed *M. sativa* hay (16.4% CP, 59.4% NDF and 57.9% IVDOM) mixed with either *D. cinerea* or *A. nilotica* seeds. The other group of 10 animals was also divided similarly and each animal of this group was fed *D. eriantha* hay (5.5% CP, 72.0% NDF and 54.3% IVDOM) mixed with either *D. cinerea* or *A. nilotica* seeds.

### **3.3.5 Seed Recovery from Feces**

All experimental animals in experiment one and two were allowed to consume seeds within 24 h, after which the remaining seeds were collected and counted. Fecal collection commenced after

the end of the 24 h seed-feeding period and continued until no seeds were found in the feces. Feces from goats and sheep were collected from the fecal bags, whereas feces from cattle were collected from the concrete floor once a day for the duration of the trial. Feces were immersed in cold water until soft and then washed with tap water through a wire strainer until the water was clear. A cabinet with a light source below a glass surface was used to separate seeds from fecal remains. Seeds retrieved from each animal for the day were counted and stored in brown bags in a cool dry place pending the germination trial.

### **3.3.6 Germination Tests**

Germination tests were done at the Agricultural Research Council, Roodeplaat Forage Genebank (International Seed Testing Association 1985). Germination tests used a completely randomized experimental design. Petri dishes containing one disk of germination paper and 5 ml of distilled water were used for the germination test. Each petri dish contained a maximum of 50 seeds from each animal for each day. Germination tests were run in germination chambers kept at 20-30 °C with a 16 h dark period and 8 h light period. The germination trial was monitored daily for 21 d, and all germinated seeds were recorded. The percentage germination was calculated at the end of germination tests as the number of seeds germinated divided by the total number of seeds placed in a petri dish multiplied by 100 (Armke and Scott 1999). All seeds that did not germinate at the end of 21 d were counted and subjected to a viability test. *D. cinerea* and *A. nilotica* seeds were scarified using sand paper and soaked in distilled water for 18 h. Seeds were soaked in 1% Tetrazolium solution (2,3,5-triphynyl chloride) for 18 h in an incubator at 30°C. Each seed was cut longitudinally through the endosperm to expose the embryo through a microscope to evaluate viability (International Seed Testing Association 2012).

### 3.3.7 Statistical Analysis

The experiments were done at different times, due to the limited availability of seeds. Hence, the analyses were done separately. Each animal in experiment one (goats and sheep) and experiment two (goats and cattle) ( $n = 20$  of each animal species) was considered an experimental unit. Differences between means were considered significant at 5% level using Fisher's protected t-LSD (least significant difference). The standardized residuals were tested for normality and homogeneity of variance using Shapiro-Wilks test (1965). All data analyses were done using SAS statistical software (SAS Institute 2002) for a completely randomized design.

## 3.4. Results

### 3.4.1 Seed size, density and nutritive value

The length and width of *D. cinerea* seeds were  $4.2 \pm 0.1$  mm and  $1.7 \pm 0.1$  mm and for *A. nilotica* seeds were  $6.9 \pm 0.1$  mm and  $5.7 \pm 0.1$  mm. The density of *A. nilotica* seeds ( $1.6 \text{ g cm}^{-3} \pm 0.01$ ) was lower than that of *D. cinerea* seeds ( $1.7 \text{ g cm}^{-3} \pm 0.07$ ). The two seed species (*D. cinerea* and *A. nilotica*) used in both experiments (goats, sheep and cattle, goats) have relatively high crude protein (CP), with *D. cinerea* having a higher mean value ( $19.3\% \pm 0.06$ ) than *A. nilotica* ( $14.1\% \pm 0.06$ ). *Dichrostachys cinerea* seeds ( $15.7 \text{ MJ/kg} \pm 0.20$ ) had slightly less energy than *A. nilotica* seeds ( $16.5 \text{ MJ/kg} \pm 0.12$ ).



### 3.4.2 Seed Consumption and Recovery

#### 3.4.2.1 Experiment one (goats and sheep)

Both goats and sheep consumed all 1000 *D. cinerea* seeds offered. However, sheep consumed more ( $P < 0.001$ ) *A. nilotica* seeds ( $940 \pm 17.24$  seeds) than goats did ( $628 \pm 26.15$  seeds). There were no interactions or main effects for cumulative percentage viable seed recovery ( $P > 0.05$ ) in respect to animal and seed species. However, sheep and goats had a higher ( $P < 0.05$ ) cumulative percentage seed recovery when they ate the high-quality hay ( $47.40\% \pm 4.65$ ) compared to low-quality hay ( $30.21\% \pm 3.24$ ) ( $P < 0.01$ ; Fig. 1).

#### 3.4.2.2 Experiment two (goats and cattle)

Cattle consumed more ( $1000 \pm 0.00$  seeds;  $P < 0.05$ ) *A. nilotica* seeds than goats ( $820 \pm 25.12$ ). There were no significant differences ( $P > 0.05$ ) in the number of *D. cinerea* seeds consumed by cattle ( $1000 \pm 0.00$ ) than by goats ( $961 \pm 4.78$ ). No significant effects ( $P > 0.05$ ) of diet, seed species and the interaction of diet, seed species and animal species were found on cumulative percentage recovery. However, more ( $P < 0.05$ ) seeds were retrieved from cattle than from goats (cattle  $50.29\% \pm 4.27$ ; goats  $31.98\% \pm 6.44$ ; Fig. 2).

### 3.4.3 Germination Trial

#### 3.4.3.1 Experiment one (goats and sheep)

There were more viable *A. nilotica* and *D. cinerea* seeds (83.94% and 92.17%, respectively) than dead seeds after 21 days of seed germination tests. The interaction effect of animal species, diet quality and seed species had a significant effect on cumulative percentage germination ( $P < 0.0001$ ).

For goats fed low-quality hay, *A. nilotica* seed germination ( $9.38\% \pm 3.66$ ) was higher ( $P < 0.05$ ) than *D. cinerea* seed germination ( $6.78\% \pm 1.13$ ). A greater germination percentage of *D. cinerea* seeds ( $6.71\% \pm 1.53$ ) was observed in goats fed high-quality diet than for *A. nilotica* seeds ( $2.50\% \pm 0.97$ ;  $P < 0.05$ ).

In sheep, germination percentages for animals fed low-quality hay and *D. cinerea* seeds ( $10.58\% \pm 1.84$ ) were higher ( $P < 0.05$ ) than for sheep fed low-quality hay and *A. nilotica* seeds ( $5.42\% \pm 1.12$ ; Figure 3). There were no significant differences ( $P > 0.05$ ) in germination percentages between sheep fed high-quality hay and *A. nilotica* versus *D. cinerea* seeds (Figure 3).

#### 3.4.3.2 Experiment two (goats and cattle)

There were more viable *A. nilotica* and *D. cinerea* seeds (64.74% and 57.7%) than dead seeds after 21 d of seed germination tests in this experiment. Animal species was the only significant effect on mean cumulative germination percentage (goats  $14.07\% \pm 1.48$ ; cattle  $9.33\% \pm 0.94$ ) ( $P < 0.01$ ). There were no significant differences ( $P > 0.05$ ) in germination percentages between *A. nilotica* seeds and *M. sativa* hay fed to the goats and *D. cinerea* seeds and *M. sativa* hay fed to

the goats. In cattle, there were no significant differences ( $P > 0.05$ ) in germination percentages between *A. nilotica* seeds and *D. eriantha* hay or between *D. cinerea* seeds and *D. eriantha* hay. The same absence of a significant pattern ( $P > 0.05$ ) occurred for *A. nilotica* seeds and *M. sativa* hay and *D. cinerea* seeds and *M. sativa* hay when ingested by cattle.

### **3.5. Discussion**

#### **3.5.1 Seed Recovery and Germination after Ingestion**

The morphology of the two seed species (*D. cinerea* and *A. nilotica*) may explain the consumption, seed recovery and germination percentages obtained in this study. The two seed species differed in size and hardness, with *D. cinerea* being smaller and harder than *A. nilotica*. *D. cinerea* seeds may have been easier to swallow with less chewing due to their relatively small size compared to *A. nilotica* seeds. The higher crude protein (despite lower energy levels) in *D. cinerea* seeds than in *A. nilotica* seeds may have been a reason why this seed species was consumed to a greater degree. Furthermore, the larger the animal the less likely it is to be a selective feeder (van Soest 1994). Cattle are bulk feeders, which may be the reason why all *D. cinerea* and *A. nilotica* seeds in experiment two (goats vs. cattle) were consumed irrespective of seed size.

Small seeds of <2.5 mm in width are more likely to escape mastication and rumination better than large seeds (Russi et al. 1992; Gardener et al. 1993; Whitacre and Call 2006). In addition, if small food particles are able to pass through the reticulo-rumen orifice to the lower parts of the digestive tract faster than large particles (Minson 1990), then small seeds may also pass faster (Bodmer and Ward 2006; Whitacre and Call 2006). Hard-coated seeds usually have high seed recovery due to their resistance to damage during chewing and rumination (Gardener

et al. 1993; Whitacre and Call 2006), although this may depend on the herbivore species (Rohner and Ward 1999). Hard-coated seeds have higher chances of passing through the gut without substantial damage to the seeds than soft-coated seeds (Brown and Archer 1987; Archer and Pyke 1991; Gardener et al. 1993; Miller 1995; Tjelele et al. 2012). Despite the fact that the density of *A. nilotica* seeds was lower than that of *D. cinerea* seeds, its density was still higher than some legumes such as *Trifolium semipilosum* and *Stylosanthes hamate* (Simao Neto et al. 1987). We noted that seeds may be sufficiently hard-coated that imperfect seed scarification and low germination percentages result (see also Simao Neto and Jones 1986; Simao Neto et al. 1987; Schupp et al. 1997).

Seed recovery and survival after passage through the gut depends on hardness and size of the seeds, number of seeds consumed and animal species and size (Gardener et al. 1993; Bodmer and Ward 2006; Varela and Bucher 2006; Whitacre and Call 2006; Castro et al. 2008). These factors may singly or jointly influence seed recovery and germination. The quality of the associated diet is one of the most important determinants of success of livestock fecal seed dispersal (Simao Neto et al. 1987; Miller and Coe 1993; Miller 1995; Whitacre and Call 2006). Seeds ingested when animals are consuming higher quality forage tend to pass faster through the digestive tract with less damage to the seed coat (Jones and Simao Neto 1987; Shayo and Uden 1989; Whitacre and Call 2006). Consequently, such seeds tend to germinate better than seeds that reside in the gut for longer and are further damaged by digestive juices. This may explain the significant effect of diet observed on cumulative percentage seed recovery in this experiment.

The high mean cumulative percentage seed recovery for cattle (50.3%) compared to the 32.0% for goats in experiment two may be attributed to the large difference in size of the animals. Cattle in this study conferred potentially higher seed recovery of *D. cinerea* and *A.*

*nilotica* seeds compared to goats and are therefore more likely to promote woody plant encroachment, provided that the seeds are adequately scarified. Miller (1995) and Bodmer and Ward (2006) found that seed survival through the digestive tract and seed germination increased linearly with body size. Goats and sheep chew food thoroughly (Shayo and Uden 1989; McGregor and Whiting 2013) which may result in seed damage and reduced seed recovery. The retention time of feeds in the gastro-intestinal tract of goats is shorter than that of sheep, resulting in greater passage rates (Huston et al. 1986, McGregor and Whiting 2013). However, some seeds may escape damage from chewing and rumination (Jones and Simao Neto 1987; Thompson et al. 1990; Miller 1995). Furthermore, Simao Neto and Jones (1987) and Thompson et al. (1990) have shown that large differences in seed recovery and seed germination between cattle and small ruminants (goats and sheep) may be related to initial mastication and rumination. Even though seed type did not significantly affect seed recovery, the relatively high seed recovery may be in part a function of the hard coats of both *A. nilotica* and *D. cinerea* seeds compared to other legume species previously recorded (Brown and Archer 1987; Simao Neto et al. 1987; Miller 1995; Shayo and Uden 1998).

The relatively high viability of *A. nilotica* and *D. cinerea* seeds from experiment one (83.9%; 92.2%) and experiment two (64.7; 57.7%) shows that passage through the digestive system of these livestock did not greatly compromise germination potential. However, under natural conditions, seed dispersal does not guarantee seed germination, which depends on appropriate environmental and seed survival conditions (Ward and Rohner 1997; Rohner and Ward 1999; James et al. 2011; Grellier et al. 2012).

### 3.6. Management Implications

In experiment one (goats and sheep); the effect of diet quality influenced both seed recovery and germination percentages in goats and sheep. This suggests that animals fed seeds mixed with high-quality diet are most likely to pass through the digestive tract intact and remain viable. Results from experiment two also showed that more seeds were retrieved from cattle than goats, which is attributed to animal body size and quality of diet consumed, both of which influence retention time in the gut (Miller 1995; Bodmer and Ward 2006). The relatively high seed recovery and germination percentages in this study may be due to the morphology (size, hardness) of the two seed species (Whitacre and Call 2006; Tjelele et al. 2012).

*Acacia nilotica* and *D. cinerea* seeds remained in the gut of goats, sheep and cattle for about 9 d in this study. Thus, seeds can potentially germinate in grazing areas. The interaction of animal species and size, diet quality, and seed characteristics (size, hardness) all played an important role in recovery of viable and scarified seeds either alone or in combination. Animals consuming *D. cinerea* and *A. nilotica* seeds during the dry season should be restricted to fenced paddock/s to reduce the possibility of woody plant encroachment.

### 3.7. Acknowledgements

We thank Thamsanqa Mpanza, Lekukela Mohale, Malose Matlou, Marvin Mavhunga and Slindokuhle Khumalo for their assistance during the experiment. The financial support of Gauteng Department of Agriculture and Rural Development (GDARD), National Research Foundation (NRF) and International Foundation for Science (IFS) is appreciated. We thank

Frikkie Calitz and Eric Mathebula for assistance with statistical analysis. We are grateful to Department of Agriculture, Forestry and Fisheries (DAFF) staff (particularly Obed Phahladira) for their help with viability tests.

### 3.8. Literature Cited

- Association of Official Analytical Chemists. 2000. Official methods of analyses. 17<sup>th</sup> ed. Inc., Arlington, Virginia, USA.
- Archer, S., and D.A. Pyke. 1991. Plant-animal interaction affecting plant establishment and persistence on revegetated rangelands. *Journal of Range Management* 44:558-565.
- Armke, F.W., and C.B. Scott. 1999. Using cattle to disperse seeds for winter forage plants. *Texas Journal of Agriculture and Natural Resources* 12:28-38.
- Bodmer, R.E., and D. Ward. 2006. Frugivory in large mammalian herbivores. *In*: K. Danell, P. Duncan, R. Bergstrom and J. Pastor [eds.]. Large herbivore ecology, ecosystem dynamics and conservation. Cambridge, United Kingdom: Cambridge University Press. p. 232-260.
- Brown, J.R., and S. Archer. 1987. Woody plant seed dispersal and gap formation in a North American subtropical savanna woodland: the role of domestic herbivores. *Plant Ecology* 73:73-80.
- Castro, S.A., F. Bozinovis, and F.M. Jaksic. 2008. Ecological efficiency and legitimacy in seed dispersal of an endemic shrub (*Lithrea caustica*) by the European rabbit (*Oryctolagus cuniculus*) in central Chile. *Journal of Arid Environments* 72:1164-1173.

- El Aich, A., and A. Waterhouse. 1999. Small ruminants in environmental conservation. *Small Ruminant Research* 34:271-287.
- Engels, E.A.N., and F.J. van der Merwe. 1967. Application of an *in vitro* technique to South African forages with special reference to the effect of certain factors on the results. *South African Journal of Agricultural Science* 10:983-992.
- Gardener, C.J., J.G. Mclvor, and A. Jansen. 1993. Passage of legume and grass seeds through the digestive tract of cattle and their survival in feces. *Journal of Applied Ecology* 30:63-74.
- Grellier, S., S. Barot, J.L. Janeau, and D. Ward. 2012. Grass competition is more important than seed ingestion by livestock for *Acacia* recruitment in South Africa. *Plant Ecology* 213: 899-908.
- Hoffman, M.T., R.M. Cowling, C. Douie, and S.M. Pierce. 1989. Seed predation and germination of *Acacia erioloba* in the Kuiseb River Valley, Namib Desert. *South African Journal of Botany* 55:103-106.
- Huston, J.E., B.S. Rector., W.C. Ellis, and M.L. Allen. 1986. Dynamics of digestion in cattle, sheep, goats and deer. *Journal of Animal Science* 62:208-215.
- International Seed Testing Association. 1985. International rules for seed testing. *Seed Science and Technology* 13:299-355.
- International Seed Testing Association. 2012. International rules for seed testing. ISTA, Bassersdorf, Switzerland.



- James, J.J., T.J. Svejcar, and M.J. Rinella. 2011. Demographic processes limiting seedling recruitment in arid grassland restoration. *Journal of Applied Ecology* 48:961-969.
- Janzen, D.H. 1984. Dispersal of small seeds by big herbivores: foliage is the fruit. *American Naturalist* 123:338-353.
- Jones, R.M., and M. Simao Neto. 1987. Recovery of pasture seed ingested by ruminants. 3. The effect of the amount of seed in the diet and of diet quality on seed recovery from sheep. *Australian Journal of Experimental Agriculture* 27:253-256.
- McGregor, B.A., and C.J. Whiting. 2013. Grain excretion by goats fed whole or processed cereals with various roughages. *Small Ruminant Research* 115:21-28.
- Miller, M.F., and M. Coe. 1993. Is it advantageous for *Acacia* seeds to be eaten by ungulates? *Oikos* 66:364-368.
- Miller, M.F. 1995. *Acacia* seed survival, seed germination and seedling growth following pod consumption by large herbivores and seed chewing rodents. *African Journal of Ecology* 33:194-210.
- Minson, D.J. 1990. Forage in ruminant nutrition. Academic Press, San Diego, CA, USA.
- Myers, J.A., M. Vellend, and S. Gardescu. 2004. Seed dispersal by white-tailed deer: implications for long distance dispersal, invasion, and migration of plants in eastern North America. *Oecologia* 139:35-44.
- Nathan, R., and H.C. Muller-Landau. 2000. Spatial patterns of seed dispersal, their determinants and consequences for recruitment. *Trends in Ecology and Evolution* 15:278-285.

- Or, K., and D. Ward. 2003. Three-way interaction between acacias, large mammalian herbivores and bruchid beetles - a review. *African Journal of Ecology* 41:257-265.
- Pakeman, R. J., G. Digneffe, and J.L. Small. 2002. Ecological correlates of endozoochory by herbivores. *Functional Ecology* 16:296-304.
- Robbins, C.T. 1993. Wildlife feeding and nutrition. 2<sup>nd</sup> ed. Academic Press, New York, USA.
- Rohner, C., and D. Ward. 1999. Large mammalian herbivores and the conservation of arid *Acacia* stands in the Middle East. *Conservation Biology* 13:1162-1171.
- Russi, L., P.S. Cocks, and E.H. Roberts. 1992. The fate of legume seeds eaten by sheep from a Mediterranean grassland. *Journal of Applied Ecology* 29:772-778.
- SAS Institute. 2002. SAS. Version 9.2. SAS Institute, Cary, NC, USA.
- Schupp, E.W., J.M. Gomes., J.E. Jimenez, and M. Fuentes. 1997. Dispersal of *Juniperus occidentalis* (Western Juniper) seeds by frugivorous mammals on Juniper Mountain, southeastern Oregon. *Great Basin Naturalist* 57:74-78.
- Shapiro, S.S., and M.B. Wilks. 1965. An analysis of variance test for normality (complete samples). *Biometrika* 52:591-611.
- Shayo, C.M., and P. Uden. 1998. Recovery of seed of four African browse shrubs ingested by cattle, sheep and goats and the effect of ingestion, hot water and acid treatment on the viability of the seeds. *Tropical Grasslands* 32:195-200.
- Simao Neto, M., and R.M. Jones. 1986. The effect of storage in cattle dung on viability of tropical pasture seeds. *Tropical Grasslands* 20:132-135.

- Simao Neto, M., and R.M. Jones. 1987. Recovery of pasture seed ingested by ruminants. 2. Digestion of seed in nylon bags and *in vitro*. *Australian Journal of Experimental Agriculture* 27:239-246.
- Simao Neto, M., R.M. Jones, and D. Ratcliff. 1987. Recovery of pasture seed ingested by ruminants. 1. Seed of six tropical pasture species fed to cattle, sheep and goats. *Australian Journal of Experimental Agriculture* 27:239-246.
- Thompson, E.F., S. Rihawi., P.S. Cocks., A.E. Osman, and L. Russi. 1990. Recovery and germination rates of seeds from Mediterranean medics and clovers offered to sheep at a single meal or continuously. *Journal of Agricultural Science* 114:295-299.
- Tilley, J.M.A., and Terry, R.A. 1963. A two-stage technique for *in vitro* digestion of forage crops. *Journal of the British Grassland Society* 18:104-111.
- Tjelele, T.J., L.E. Dziba, and H.T. Pule. 2012. Recovery and germination of *Dichrostachys cinerea* seeds fed to goats (*Capra hircus*). *Rangeland Ecology and Management* 65:105-108.
- van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods of dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74:3583-3597.
- van Soest, P.J. 1994. Nutritional ecology of the ruminant. 2<sup>nd</sup> ed. Cornell University Press, Ithaca, New York, USA.

Varela, O., and E.H. Bucher. 2006. Passage time, viability, and germination of seeds ingested by foxes. *Journal of Arid Environments* 67:566-578.

Ward, D., and C. Rohner. 1997. Anthropogenic causes of high mortality and low recruitment in three *Acacia* tree taxa in the Negev desert, Israel. *Biodiversity and Conservation* 6:877-893.

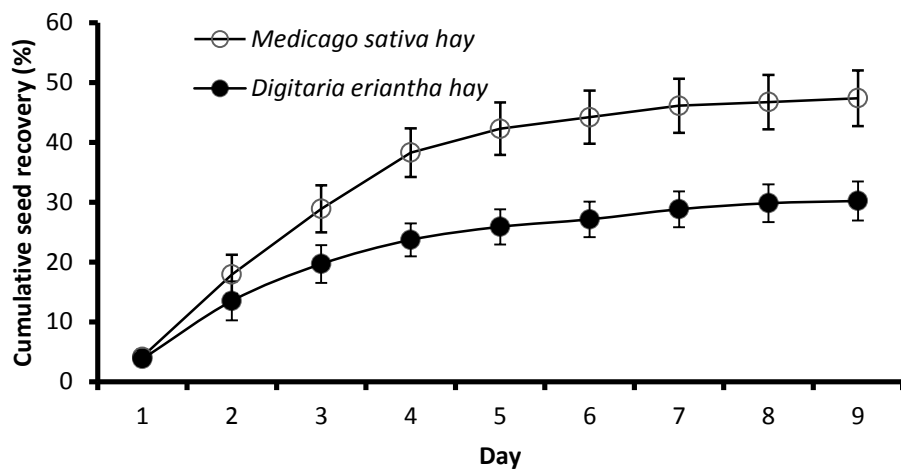
Whitacre, M.K., and C.A. Call. 2006. Recovery and germinability of native seed fed to cattle. *Western North American Naturalist* 66:121-128.

### 3.9. Figure Legends

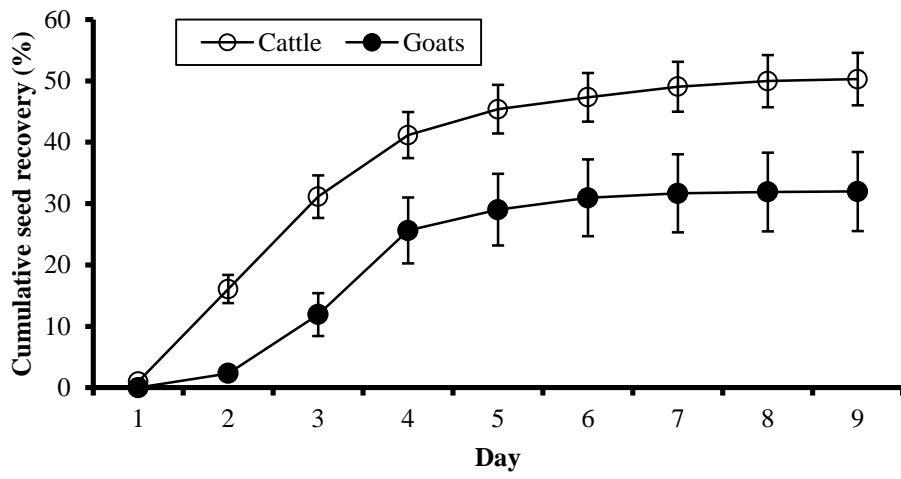
**Figure 1.** Significant effect of diet (high-quality hay and low-quality hay) on mean cumulative percentage seed recovery from experiment one. Bars represent standard errors (S.E). Fisher's *post hoc* test was used.

**Figure 2.** Significant effect of animal species on mean cumulative percentage seed recovery from experiment two. Bars represent standard errors (S.E). Fisher's *post hoc* test was used.

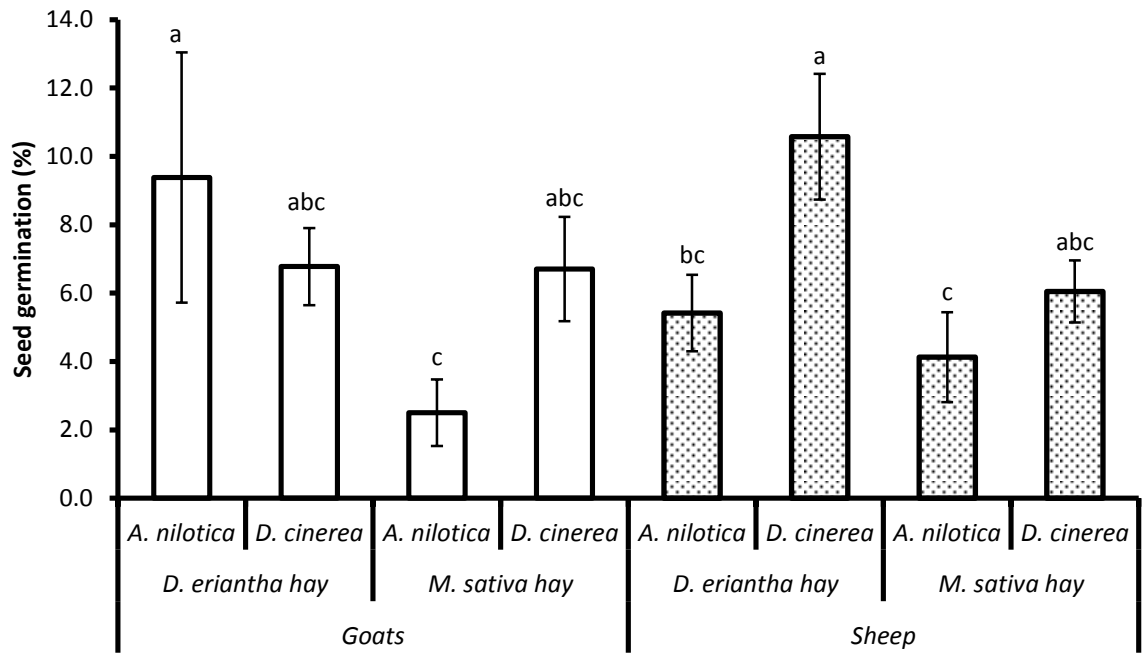
**Figure 3.** Interaction effect of animal species (goats, sheep), diet (*D. eriantha* hay, *M. sativa* hay) and seed species (*A. nilotica*, *D. cinerea*) on percentage seed germination. Bars represent standard errors (S.E). Fisher's *post hoc* test was used.



**Figure 1.**



**Figure 2.**



**Figure 3.**



## Chapter 4

### **The effects of gut passage and dung fertilization on seedling establishment of *Dichrostachys cinerea* and *Acacia nilotica***

TJ Tjelele<sup>1,2\*</sup>, D Ward<sup>2</sup> and LE Dziba<sup>3</sup>

<sup>1</sup>Agricultural Research Council - Animal Production Institute, Rangelands Management, Irene, 0062, South Africa; <sup>2</sup>College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Scottsville 3209, South Africa; <sup>3</sup>CSIR: Natural Resources and the Environment, Pretoria 0001, South Africa

Correspondence author: Julius Tjelele, Agricultural Research Council - Animal Production Institute, Rangelands Management, Private Bag X2, Irene, 0062, South Africa. Email: [jtjelele@arc.agric.za](mailto:jtjelele@arc.agric.za)

**Formatted for submission to *African Journal of Range and Forage Science***

#### 4.1. Abstract

*Dichrostachys cinerea* and *Acacia* species pods have higher nutritive value than grasses and other browse plants during the dry season and form an important part of the diet of livestock. Ingested seeds of these species pass through the digestive tract of livestock and usually remain viable even after mechanical (chewing) and chemical (digestive) scarification. We studied seedling emergence, seedling establishment and recruitment of *D. cinerea* and *A. nilotica* seeds dispersed by cattle and goats under natural conditions. Significantly more *A. nilotica* and *D. cinerea* seeds were retrieved from cattle ( $40.0\% \pm 3.6$ ;  $25.7\% \pm 3.9$ ) than goats ( $11.7\% \pm 3.1$ ;  $13.2\% \pm 3.8$ ) for both tree species, respectively. However, there was a significant interaction between animal species (goats, cattle) and other factors such as seed recovery day, seed germination treatment (seeds placed on top of the soil with no dung, seeds buried 2 cm under the soil with no dung, seeds buried 2 cm under the soil with dung) and season (dry, wet) on percentage seedling recruitment. Most importantly, seeds retrieved from goats ( $11.96\% \pm 0.06$ ) recruited significantly better than seeds retrieved from cattle ( $7.62\% \pm 0.05$ ) and control/untreated seeds (i.e. no passage through the gut) ( $4.12\% \pm 0.02$ ). More seeds can potentially germinate and recruit following seed ingestion by goats than cattle and untreated seeds. Goats and cattle may facilitate woody plant encroachment by enhancing seedling emergence.

**Keywords:** dung nutrients, recruitment, scarification, seedling emergence, woody plant encroachment

## 4.2. Introduction

*Dichrostachys cinerea* and *Acacia* species pods have higher nutritive value than grasses and other browse plants, especially during the dry season (Tanner et al. 1990). They form an important part of the diet of livestock in many African countries. A considerable amount of seeds of various *Acacia* species and *D. cinerea* ingested by browsing or grazing animals pass through the digestive tract via the faeces (Halevy 1974, Tanner et al. 1990, Shayo and Uden, 1998, Tjelele et al. 2012). Seeds that retain viability after passage through the digestive tract could be dispersed by animals over a large area and subsequently establish, facilitating rapid woody plant encroachment (Simao Neto et al. 1987, Gibbens et al. 1992, Lerner and Peinetti 1996, Cuda and de Loach 1998, Rohner and Ward 1999, Pakeman et al. 2009, Carlo et al. 2011).

Woody plant encroachment (i.e. an increase in density of woody plants) is one of the major challenges to livestock production in southern Africa because it reduces carrying capacity by limiting production of the herbaceous understory and access to quality forage (Schupp et al. 1997, Smit 2004, Ward 2005). Several causes of encroachment have been studied, which among other factors include enhanced animal seed dispersal, plant chemical defence, reduced fire frequency and global climate change (Trollope 1980, O'Connor 1996, Rohner and Ward 1999, Sankaran et al. 2004). Most of these studies suggest that multiple factors are ultimately responsible for woody plant encroachment and have, therefore, been inconclusive on the causes of woody plant encroachment. This poses serious challenges for management of woody plant encroachment (Scholes and Archer 1997, Ward 2005). Adaptive management requires understanding the causes of woody plant encroachment to facilitate changes in effective management policies (Ward 2005).

It is clear that precipitation, fire, herbivory and soil nutrients are major determinants of tree-grass balance in savannas (Sankaran et al. 2004, Ward 2005, Kraaij and Ward 2006, Wiegand et al. 2006). Other factors such as seed gut passage and seed germination (Or and Ward 2003, Tjelele et al. 2012) and bruchid beetles (Coe and Coe 1987, Or and Ward 2007) have also been considered to affect tree population dynamics. Herbivores may reduce grass biomass, reducing competition with trees (Kraaij and Ward 2006, Goheen et al. 2010), and may also increase seed germination, survival and recruitment of woody plants through seed scarification and dung fertilization (Halevy 1974, Miller and Coe 1993, Anderson 2001, Or and Ward 2003, Bodmer and Ward 2006).

Studies of seed recovery, seed germination, seedling establishment and recruitment in savanna ecosystems are limited. Savanna ecosystems are characterised by the co-dominance of two contrasting plant life forms, i.e. trees and grasses (Sankaran et al. 2004). Despite their recognised importance to human welfare, the dynamics of savannas are poorly understood (Jeltsch et al. 1998). Particularly, the mechanisms that allow trees and grasses to coexist and factors determining the relative proportions of woody and herbaceous components remain unclear (Scholes and Archer 1997, Ward 2005, Bond 2008).

The appearance of a radicle marks the end of seed germination (the emergence and development from the seed embryo) and the beginning of seedling establishment, a period that ends when the seedling has exhausted the food reserves stored in the seed (Westoby et al. 1996, Weitbrecht et al. 2011). Seed germination and seedling establishment stages are critical and vulnerable to water stress and injury (Meyer and Pendleton 2005). Seedling recruitment refers to the process by which new individuals/seedlings establish a new population or are added to an existing population (Herrera et al. 1994, Schupp and Fuentes 1995). Tree seedling recruitment is

limited by supply of seeds and the availability of suitable sites for a particular seed species to germinate and establish (Clark et al. 1998, Caspersen and Saprunoff 2005, Burkart et al. 2010). Favourable conditions for seedling establishment are highly variable on most rangelands (Fuhlendorf 1999). When conditions are appropriate for seed to germinate, seedlings may not survive the dry season or even the wet season (O'Connor 1995). In addition, seed mass may influence aspects of plant ecology (Moles et al. 2005). Small-seeded species are able to produce more seeds than large-seeded species (Henery and Westoby 2001); whereas seedlings from large-seeded species may tolerate stress during establishment (Leishman et al. 2000). This suggests that seeds retrieved from herbivores are not guaranteed to germinate; and seedling establishment and recruitment, which are pre-requisites for maintaining or increasing tree abundance and are influential in woody plant encroachment, are not assured (Nathan and Muller-Landau, 2000, Kraaij and Ward 2006). A critical point or threshold is reached once plants become well established, increase their growth rate and reach the seed-producing stage (Fuhlendorf et al. 1996).

To better understand the mechanisms involved in woody plant encroachment, we studied seedling emergence and seedling recruitment of *D. cinerea* and *A. nilotica* seeds dispersed by cattle and goats under natural conditions. We predicted that 1) seedling emergence and seedling establishment will be favoured by passage through the digestive tract of goats and cattle and dung fertilization (nutrient input), and 2) seedling survival and recruitment will increase during the rainy season relative to the dry season.

### **4.3. Materials and methods**

#### ***4.3.1. Study area***

The study was done at the Agricultural Research Council (ARC) Roodeplaat Experimental Farm, Gauteng Province, South Africa (28°19'E, 25°35'S). The natural vegetation component of the farm used for livestock production and game encompasses an area of approximately 2100 ha. The vegetation type of Roodeplaat is classified as Marikana Thornveld by Mucina and Rutherford (2006), and is generally characterized by open *Acacia karroo* and *A. caffra* woodlands occurring in the valleys (Mucina and Rutherford 2006). Shrubs are more dense along drainage lines and rocky outcrops.

We chose *A. nilotica* and *D. cinerea* seeds because of their different seed characteristics (size, shape and hardness) (among other reasons) as well as the extent of encroachment of the two species in areas where most of the emerging farmers are situated. The mean annual rainfall is 646 mm, and the minimum and maximum summer and winter temperatures are 20–29 °C and 2–16 °C, respectively.

#### ***4.3.2. Seed collection***

*Dichrostachys cinerea* and *Acacia nilotica* seeds were purchased from Silver Hills Seeds Company in Kenilworth, Cape Town, South Africa. The seeds were immersed in water and any floating seeds were discarded because they were either unripe or damaged by bruchid beetles (Or and Ward 2003). The viability test of *D. cinerea* and *A. nilotica* seeds prior to feeding the animals showed the seeds were 95 and 91% viable for each species, respectively.

#### **4.3.3. Experimental animals**

Fourteen Bonsmara cows (mean  $\pm$  SE), 218.43 kg  $\pm$  3.00)) and 14 female indigenous goats (23.70 kg  $\pm$  0.62) were used during the trial. Seven paddocks of 0.5 ha in size (seven replicates) were fenced at ARC Roodeplaat Experimental Farm, where each paddock had two Bonsmara cows and two female indigenous goats (i.e. each paddock had four animals). One cow was gavaged or force-fed with 1500 *D. cinerea* seeds and the other cow gavaged with 1500 *A. nilotica* seeds. One goat was also gavaged with 1500 *D. cinerea* seeds and the other goat gavaged with 1500 *A. nilotica* seeds and randomly kept in a paddock. All goats were fitted with faecal bags for easy collection of faeces. Due to the unavailability of faecal bags for cows, cattle dung pats were collected from the ground in each paddock. All animals were offered clean water throughout the experiment.

#### **4.3.4. Recovery of seeds from faeces**

Faeces of both animal species were collected daily for the duration of the experiment. Faeces were immersed in cold water until soft and then washed with tap water through a wire strainer until the water was clear. Faecal collection commenced 24 h after force-feeding and continued until no seeds were found in the faeces (about seven days). A cabinet with a light source below a glass surface was used to separate seeds from fecal remains. Retrieved undamaged seeds from the dung for each goat and each cow for that day were counted and stored in brown paper bags in a cool dry place pending the germination trial.

#### **4.3.5. Seedling emergence, seedling establishment and recruitment**

Seedling emergence tests were conducted at the Agricultural Research Council's Roodeplaat Farm. Seedling emergence potential was determined for seeds subjected to the following: 1) control (untreated seeds (i.e. not ingested)), 2) seeds retrieved from cattle faeces, and 3) seeds retrieved from goat faeces. Seeds retrieved during the first three days (F3) and the last four days (L4) (i.e. a total of seven days of seed recovery) from goats and cattle were planted separately. Seeds retrieved during the first three days usually germinate less than seeds retrieved from the fourth day (Tjelele et al. 2012), as a result of time spent in the gut. Retrieved seeds from goats and cattle during the first three days and the last four days were subjected to the following planting methods: 1) seeds placed on top of the soil with no dung, 2) seeds buried 2 cm in the soil with no dung (ND) and 3) seeds buried 2 cm in the soil with dung (WD). Untreated/control seeds (i.e. not ingested) were also subjected to the following planting methods: 1) seeds placed on top of the soil with no dung, 2) seeds buried 2 cm in the soil with no dung, and 3) seeds buried 2 cm in the soil with dung. Each of the planting methods had two replicates of 25 seeds, i.e. seeds retrieved from goats and cattle during the first three days and the last four days and untreated seeds. Seedling emergence, seedling establishment and seedling survival were monitored over three periods in two seasons (May 2011- September 2011, dry season), (October 2011-April 2012, wet season), and (May 2012-July 2012, dry season). No watering of seeds was applied throughout the trial; seeds relied only on rainfall (576.6 mm; Figure 1).

The experiment lasted for a year. The seeds were planted in May 2011, the beginning of the dry season. This is the time when animals consume and disperse seeds of different woody plant species including *D. cinerea* and *A. nilotica* seeds. Seeds were monitored monthly to record the number of seedling emergence and seedling survival (survival was measured from the



beginning to the end of the experiment and is defined as the proportion of emerging seedling that survived to be a recruit) until end of July 2012. Seedling recruitment was determined from the difference between the number of seeds that emerged minus the number of seedlings that died (see Grellier et al. 2012).

#### **4.3.6. Statistical analysis**

All data analyses were done using SAS statistical software (SAS Institute 2002). Seed recovery and germination tests were subjected to a completely randomized experimental design. Analysis of variance (ANOVA) was used to test the effects of animal species (goats, cattle) and control, seed species (*D. cinerea* and *A. nilotica*) and seed recovery days (F3 = first three days and L4 = last four days) and dung (and no dung) on seed germination, seedling survival and recruitment after a Logit transformation (Snedecor & Cochran 1967) with normal residuals and homogeneity of variance. The same plants were repeatedly measured every month for the duration of the experiment. Repeated measurements were included in the analysis as a sub-plot factor (Little and Hills 1972). The percentage seed germination, seedling survival and seedling recruitment were the dependent variables. The standardized residuals were tested for normality using Shapiro-Wilks test (1965). A Bonferroni adjustment of  $\alpha = 0.017$  was used as the critical level to control for Type 1 statistical error.

### **4.4. Results**

#### **4.4.1. Seed recovery**

The interaction effect of animal species (cattle and goats), seed species (*D. cinerea* and *A. nilotica*) and seed recovery day on cumulative percentage seed recovery was significant ( $P <$

0.0001). There were significant differences in cumulative seed percentage of *A. nilotica* seeds ( $37.8\% \pm 3.9$ ) and *D. cinerea* ( $22.9\% \pm 4.3$ ) retrieved from cattle than *A. nilotica* seeds ( $11.7\% \pm 3.1$ ) and *D. cinerea* ( $13.2\% \pm 3.8$ ) retrieved from goats (Figure 2). However, there was no significant difference in cumulative percentage seeds retrieved from goats from the two seed species (*A. nilotica* and *D. cinerea*) (Figure 2).

#### **4.4.2. Seedling emergence**

There was a significant interaction effect ( $P < 0.0001$ ) of animal species (cattle and goats), seed recovery day (F3 = first three days and L4 = last four days) and planting methods/germination treatments (seed placed on top of the soil with no dung (Top), seeds buried 2 cm in the soil with no dung (ND), seeds buried 2 cm in the soil with dung (WD)) on percentage seed germination (Table 1). Seeds retrieved from goats in the last four days (L4) and planted 2 cm in the soil with dung (WD =  $30.55\% \pm 0.42$ ) had significantly higher emergence rates than seeds planted 2 cm in the soil with no dung (ND =  $24.87\% \pm 0.34$ ) and seeds planted on top of the soil with no dung (Top =  $24.24\% \pm 0.43$ ; Figure 3). Seeds retrieved from goats in the first three days and planted 2 cm in the soil with dung ( $10.04\% \pm 0.11$ ) and seeds planted 2 cm in the soil with no dung ( $10.22\% \pm 0.08$ ), emerged significantly more than seeds planted on top of the soil with no dung ( $8.49\% \pm 0.08$ ). Seeds retrieved from cattle in the last four days and planted 2 cm with no dung ( $15.97\% \pm 0.14$ ) and seeds planted 2 cm in the soil with dung ( $13.36\% \pm 0.10$ ) emerged greater than seeds planted on top of the soil with no dung ( $9.67\% \pm 0.10$ ). Seeds retrieved from cattle and planted on top of the soil with no dung ( $6.81\% \pm 0.08$ ) recruited significantly better than seeds planted 2 cm in the soil with no dung ( $4.88\% \pm 0.07$ ) and seeds planted 2 cm in the soil with dung ( $4.22\% \pm 0.04$ ). Untreated seeds (i.e. not ingested), planted with dung and planted on

top of the soil with no dung generally had lower germination than animal-ingested seeds (Figure 3).

The interaction effect of animal species and seed species had an effect on seedling emergence ( $P < 0.0001$ ; Table 1). *D. cinerea* seeds retrieved from goats ( $16.14\% \pm 0.10$ ) emerged significantly more than *A. nilotica* seeds ( $5.03\% \pm 0.05$ ). *D. cinerea* seeds retrieved from cattle ( $9.04\% \pm 0.06$ ) emerged significantly better than *A. nilotica* seeds ( $4.89\% \pm 0.03$ ) and untreated/control *A. nilotica* seeds ( $3.10\% \pm 0.02$ ).

Animal species  $\times$  seed recovery day interaction had significant effects on seedling emergence ( $P < 0.0001$ ). Seeds retrieved from goats ( $25.97\% \pm 0.22$ ) in the last four days had significantly higher emergence than seeds retrieved in the first three days ( $9.58\% \pm 0.05$ ). Seeds retrieved from goats ( $25.97\% \pm 0.22$ ) in the last four days, emerged significantly better than seeds retrieved from cattle ( $13.25\% \pm 0.07$ ) in the last four days.

The interaction effect of seed recovery day and germination treatment had a significant effect on seedling emergence ( $P < 0.0001$ ; Table 1). Seeds retrieved in the last four days and planted 2 cm in the soil with no dung ( $9.19\% \pm 0.10$ ) emerged better than seeds planted 2 cm in the soil with dung ( $8.13\% \pm 0.10$ ) and seeds planted on top of the soil with no dung ( $7.65\% \pm 0.13$ ). Seeds retrieved in the first three days and planted 2 cm in the soil with dung ( $5.12\% \pm 0.05$ ) emerged significantly less than seeds retrieved in the first three days, planted on top of the soil with no dung ( $6.07\% \pm 0.05$ ) and seeds planted 2 cm in the soil with no dung ( $5.86\% \pm 0.05$ ).

Seeds retrieved from goats ( $13.45\% \pm 0.09$ ) emerged significantly better than seeds retrieved from cattle ( $7.30\% \pm 0.05$ ) and untreated seeds ( $4.36\% \pm 0.02$ ;  $P < 0.001$ ). Seeds

retrieved in the last four days ( $8.44\% \pm 0.06$ ) from goats and cattle emerged significantly higher than seeds retrieved during the first three days ( $5.64\% \pm 0.03$ ). *D. cinerea* ( $8.02\% \pm 0.04$ ) seeds emerged significantly greater than *A. nilotica* ( $3.73\% \pm 0.02$ ) seeds. There were significant differences among the seed germination treatments/germination methods (seeds planted with no dung =  $7.22\% \pm 0.05$ , seeds planted on top of the soil with no dung =  $6.65\% \pm 0.06$ , and seeds planted with dung  $6.18\% \pm 0.05$ ).

#### ***4.4.3. Seedling survival***

The interaction effect of animal species (goats and cattle), seed recovery day (first three days and last four days) and seed germination treatment (seed placed on top of the soil with no dung, seeds buried 2 cm in the soil with no dung, seeds buried 2 cm in the soil with dung) was highly significant on percentage seedling survival ( $P < 0.0001$ ; Table 1). Seeds retrieved from goats ( $25.85\% \pm 0.46$ ) in the last four days and planted 2 cm in the soil with dung and seeds planted 2 cm in the soil with no dung ( $25.55\% \pm 0.36$ ) survived significantly better than seeds planted on top of the soil with no dung ( $16.98\% \pm 0.46$ ). However, no differences were found among seeds retrieved from goats in the first three days and planted 2 cm in the soil with dung, seeds planted 2 cm in the soil with no dung and seeds planted on top of the soil with no dung ( $P > 0.05$ ). A significantly greater seedling survival was observed on seeds retrieved from cattle in the first three days and planted 2 cm in the soil with no dung ( $15.80\% \pm 0.12$ ) and seeds planted 2 cm in the soil with dung ( $14.45\% \pm 0.10$ ) than seeds planted on top of the soil with no dung ( $6.87\% \pm 0.05$ ). Seeds retrieved from cattle in the first three days and planted on top of the soil with no dung ( $6.87\% \pm 0.05$ ) survived significantly more than seeds planted 2 cm in the soil with no dung ( $5.79\% \pm 0.08$ ) and seeds planted 2 cm in the soil with dung ( $4.39\% \pm 0.05$ ; Figure 4).

Seeds retrieved from goats ( $11.65\% \pm 0.09$ ) survived significantly better than seeds retrieved from cattle ( $8.02\% \pm 0.05$ ) and control/untreated seeds ( $4.32\% \pm 0.02$ ). Significantly more seeds retrieved in the last four days ( $8.48\% \pm 0.06$ ) survived better than seeds retrieved in the first three days ( $5.46\% \pm 0.03$ ). *D. cinerea* ( $7.75\% \pm 0.04$ ) survived more than *A. nilotica* seeds ( $3.70\% \pm 0.02$ ;  $P < 0.05$ ). Seeds planted 2 cm in the soil with no dung ( $7.39\% \pm 0.05$ ) survived significantly better than seeds planted on top of the soil with no dung ( $6.18\% \pm 0.05$ ) and seeds planted 2 cm in the soil with dung ( $5.96\% \pm 0.05$ ).

#### **4.4.4. Seedling recruitment**

The interaction effect ( $P < 0.0001$ ) of animal species (goats and cattle), seed recovery day (first three days and last four days) and seed germination treatment (seed placed on top of the soil with no dung, seeds buried 2 cm in the soil with no dung, seeds buried 2 cm in the soil with dung) was significant on seedling recruitment (Table 1). Seeds retrieved from goats in the last four days and planted 2 cm in the soil with dung ( $25.85\% \pm 0.46$ ) and seeds planted 2 cm in the soil with no dung ( $24.77\% \pm 0.35$ ) recruited significantly better than seeds planted on top of the soil with no dung ( $16.98\% \pm 0.46$ ; Figure 5). Seeds retrieved from cattle and planted 2 cm in the soil with dung ( $14.20\% \pm 0.11$ ) and seeds planted 2 cm in the soil with no dung ( $16.19\% \pm 0.14$ ) recruited significantly better than seeds planted on top of the soil with no dung ( $9.67\% \pm 0.10$ ). Significant differences were found among seeds retrieved from cattle and planted on top of the soil with no dung ( $6.58\% \pm 0.06$ ), seeds planted 2 cm in the soil with no dung ( $5.09\% \pm 0.08$ ) and seeds planted 2 cm in the soil ( $4.03\% \pm 0.04$ ; Figure 5).

Animal species and seed species interaction was significant on seedling recruitment ( $P < 0.0001$ ; Table 1). *D. cinerea* seeds retrieved from goats ( $14.11\% \pm 0.10$ ) recruited significantly

better than *A. nilotica* seeds ( $5.12\% \pm 0.06$ ). The same was true for *D. cinerea* seeds retrieved from cattle ( $9.32\% \pm 0.06$ ) and *A. nilotica* seeds ( $4.18\% \pm 0.05$ ). No significant differences were found between *A. nilotica* seeds retrieved from cattle ( $4.18\% \pm 0.05$ ) and untreated *D. cinerea* seeds ( $4.54\% \pm 0.02$ ) on seedling recruitment.

Animal species and seed recovery day had a significant interaction ( $P < 0.0001$ ) effect on seedling recruitment. Seeds retrieved from goats in the last four days ( $22.44\% \pm 0.24$ ) had a significant effect on seedling recruitment than seeds retrieved from cattle in the last four days ( $13.62\% \pm 0.07$ ). Seeds retrieved from goats in the first three days ( $8.92\% \pm 0.05$ ) recruited significantly more than seeds retrieved in the first three days from cattle ( $5.00\% \pm 0.04$ ).

Seed recovery day  $\times$  germination treatment positively affected recruitment ( $P < 0.0001$ ; Table 1). Significant differences were found among seeds retrieved in the last four days and planted 2 cm in the soil with no dung ( $8.74\% \pm 0.10$ ), seeds planted 2 cm in the soil with dung ( $7.68\% \pm 0.11$ ) and seeds planted on top of the soil with no dung ( $6.60\% \pm 0.12$ ). Seeds retrieved in the first three days, planted 2 cm in the soil with no dung ( $5.68\% \pm 0.05$ ) and seeds planted on top of the soil with no dung recruited significantly better than seeds planted 2 cm in the soil with dung ( $4.76\% \pm 0.05$ ).

Seed retrieved from goats ( $11.96\% \pm 0.06$ ) recruited significantly more than seeds retrieved from cattle ( $7.62\% \pm 0.05$ ) and control seeds ( $4.12\% \pm 0.02$ ). Significantly fewer seeds retrieved in the first three days ( $5.38\% \pm 0.03$ ) recruited better than seeds retrieved in the last four days ( $7.82\% \pm 0.06$ ). *A. nilotica* seeds ( $3.67\% \pm 0.02$ ) recruited significantly better than *D. cinerea* seeds ( $67.39\% \pm 0.04$ ). Seeds planted 2 cm in the soil with no dung ( $6.99\% \pm 0.06$ ) significantly affected seedling recruitment than seeds planted on top of the soil with no dung

(6.12%  $\pm$  0.06) and seeds planted 2 cm in the soil with dung (5.76%  $\pm$  0.05). Seedling recruitment was significantly less in the wet season (6.46%  $\pm$  0.04) than in the dry season (6.10%  $\pm$  0.05).

## **4.5. Discussion**

### ***4.5.1. Seed recovery***

The high mean cumulative percentage seed recovery of *A. nilotica* and *D. cinerea* seeds from cattle compared to goats may be attributed to body size and ingestive chewing. Seed recovery of most legume seeds depends on animal species, animal body size and hardness of the seeds (Robbins 1983, Gardener et al. 1993, Bodmer and Ward 2006, Castro et al. 2008). Bodmer and Ward (2006) found that seed survival through the digestive tract increased linearly with animal body size. The gut of big-bodied herbivores is large and seeds may have less contact with the abrasive gut surface and fewer seeds may be destroyed (Miller 1995). Small ruminants such as goats chew food more thoroughly than cattle (Shayo and Uden 1989, Tjelele et al 2012), which may result in seed damage and reduced seed recovery. However, Rohner and Ward (1999) reported that seeds ingested by ostriches had almost no surviving *Acacia* seeds because of the muscular gizzard of these birds.

### ***4.5.2. Seedling emergence, seedling survival and recruitment***

Passage of seeds through the digestive tracts of goats and cattle enhanced seedling emergence, seedling survival and seedling recruitment compared to untreated or control seeds. The hypothesis that seed germination will be favoured by acid scarification in the digestive tract of

goats and cattle (Halevy 1974, Rohner and Ward 1999, Grellier et al 2012, Tjelele et al. 2012) was supported in this study. The improvement in seed germination suggests that seed coats are adequately scarified in the gut without substantial damage to seed embryos (Brown and Archer 1987, Miller 1995, Grellier et al. 2012, Tjelele et al. 2012). Miller (1995) also reported that seed recovery and germination usually, but not always, increased with large herbivore body mass. This is probably because body mass is not the only factor, but the interaction of other factors such as the quality of diet, plant species, and seed characteristics may increase seed recovery and seedling emergence (Simao Neto et al. 1987, Whitacre and Call 2006). *A. nilotica* seeds germinated significantly lower than *D. cinerea* seeds, which was the opposite of Turnbull et al. (1999); who reported that large-seeded species are likely to show a greater seedling emergence and seedling establishment than small-seeded species.

In both goats and cattle, seeds that were retrieved during the last four days recruited significantly better than seeds retrieved during the first three days. This may be explained by the hard coat of *D. cinerea* and *A. nilotica* seeds which requires adequate scarification without substantial damage to the embryo (Brown and Archer 1987, Archer and Pyke 1991, Miller 1995, Tjelele et al. 2012).

Generally, seeds planted with no dung (either buried 2 cm in the soil or on top of the soil) germinated, survived and recruited more than seeds planted 2 cm in the soil with dung. The results were consistent with those of Grellier et al. (2012), where relatively high *Acacia sieberiana* percentage recruitment was observed with no dung (ND) than with dung, despite the application of other factors such as fire, grass competition and transit through the gut of cattle. This may be explained by the hardening of the outer layer of the seeds that had been softened (scarified) by gut passage due to exposure to the sun and dry air (see also Grellier et al. 2012,



pers. obs). The presence of decomposers in the field should decompose the dung (Brown et al. 2010), and nutrients from dung should be incorporated into the soil (Guillard 1967, Edwards and Aschenborn 1987). However, dung decomposition may have been incomplete (Grellier et al. 2012). Seeds planted with dung fertilization (i.e. with nutrient addition) were expected to have higher seedling emergence, establishment and recruitment percentages than seeds planted without dung (Mwalyosi 1990, Miller and Coe 1993, Traveset et al. 2001, Or and Ward 2003, Bodmer and Ward 2006). This was also based on the understanding that dung provides a suitable nutritive medium for seedling establishment (Coughenour and Detling 1986, Dinerstein and Wemmer 1988, Traveset and Wilson 1997).

The germination trial began during the dry season, which is the time that *D. cinerea* and *A. nilotica* seeds typically fall from trees (April-September – Coates-Palgrave 2002, Van Wyk and Van Wyk 2007). With no watering applied, this may cause the dung to turn dry and hard (Coe and Coe 1987, Miller and Coe 1993). Despite the effect of livestock on seed scarification and dung fertilization, percentage seedling recruitment was consistently higher during the wet season than the dry season. O'Connor (1995) and Wilson and Witkowski (1998) reported that rainfall is essential for seedling emergence, seedling establishment and recruitment, which may explain the results obtained in this experiment.

Clearly, this suggests that livestock play a major role in seedling recruitment through gut passage (Brown and Archer 1987, Simao Neto et al. 1987, Gibbens et al. 1992, Lerner and Peinetti 1996, Cuda and de Loach 1998, Rohner and Ward 1999, Grellier et al 2012). Seedling recruitment plays a critical role in maintaining or increasing tree abundance and is also significant in the process of woody plant encroachment (Gardener et al. 1993, Kraaij and Ward 2006, Gignoux et al. 2009).

#### **4.6. Conclusions**

Regardless of the relatively low percentage seedling emergence and seedling recruitment, it is evident that passage through the gut of livestock and longer retention time (first three days versus last four days) played a significant role. We can conclude that goats and cattle may facilitate woody plant encroachment by enhancing seed scarification. Our results also showed that dung fertilization did not improve seedling establishment and/or seedling recruitment as expected. In addition, we also emphasize the importance of precipitation on seed germination, seedling survival and recruitment (O'Connor 1995, Wilson and Witkowski 1998, Rohner and Ward 1999, Barnes 2001).

#### **4.7. Acknowledgements**

We would like to thank the following Allan Short, Malose Matlou, Marvin Mavhunga and Olga Mabelebele for their assistants during data collection. The study was funded by the Agricultural Research Council (ARC), National Research Foundation (NRF) Thuthuka PhD Track, Agricultural Sector Education Training Authority (AgriSETA) and International Foundation for Science (IFS). We further thank Biometry division of Agricultural Research Council, particularly Frikkie Calitz and Eric Mathebula for their help with statistical analysis.

#### 4.8. References

- Archer S, Pyke DA. 1991. Plant-animal interaction affecting plant establishment and persistence on revegetated rangelands. *Journal of Range Management* 44: 558-565.
- Andersen E. 2001. Effects of dung presence, dung amount and secondary dispersal by dung beetles on the fate of *Micropholis guyanensis* (Sapotaceae) seeds in central Amazonia. *Journal of Tropical Ecology* 17: 61-78.
- Barnes ME. 2001. Seed predation, germination and seedling establishment of *Acacia erioloba* in northern Botswana. *Journal of Arid Environments* 48: 541-554.
- Bodmer RE, Ward D. 2006. Frugivory in large mammalian herbivores. In: Danell K, Duncan P, Bergstrom R, Pastor J (eds), *Large Herbivore Ecology, Ecosystem Dynamics and Conservation*. Cambridge University Press, Cambridge, UK. pp 232-260.
- Bond WJ. 2008. What limits trees in C4 grasslands and savannas? *Annual Review of Ecology, Evolution and Systematics* 39: 641-659.
- Brown JR, Archer S. 1987. Woody plant seed dispersal and gap formation in a North American subtropical savanna woodland: the role of domestic herbivores. *Plant Ecology* 73: 73-80.
- Brown J, Scholtz CH, Janeau J-L, Grellier S, Podwojewski P. 2010. Dung beetles (Coleoptera: Scarabaeidae) can improve soil hydrological properties. *Applied Soil Ecology* 46: 9-16.
- Burkart M, Alsleben K, Lachmuth S, Schumacher J, Hofmann R, Jeltsch F, Schurr F. 2010. Recruitment requirements of the rare and threatened *Juncus astratus*. *Flora* 205: 583-589.

- Carlo TA, Campos-Arceiz A, Steele MA, Xiong W. 2011. Frugivory and seed dispersal integrating patterns, mechanisms and consequences of a key animal-plant interaction. *Integrative Zoology* 6: 165-167.
- Casperson JP, Saprunoff M. 2005. Seedling recruitment in a northern temperate forest: the relative importance of supply and establishment limitation. *Canadian Journal of Forest Research* 35: 978-989.
- Castro SA, Bozinovis F, Jaksic FM. 2008. Ecological efficiency and legitimacy in seed dispersal of an endemic shrub (*Lithrea caustica*) by the European rabbit (*Oryctolagus cuniculus*) in central Chile. *Journal of Arid Environments* 72: 1164-1173.
- Coates-Palgrave M. 2002. *Trees of southern Africa* (3<sup>rd</sup> ed.). Struik, Cape Town, South Africa.
- Coe M, Coe C. 1987. Large herbivores, *Acacia* trees and bruchid beetles. *South African Journal of Science* 83: 624-635.
- Coughenour MB, Detling JK. 1986. *Acacia tortilis* seed germination responses to water potential and nutrients. *African Journal of Ecology* 24: 203-205.
- Clark JS, Macklin E, Wood L. 1998. Stages and spatial scales of recruitment in southern Appalachian forests. *Ecological Monographs* 68: 213-235.
- Cuda JP, de Loach CJ. 1998. Biology of *Mozena obtuse* (Hemiptera: Coreidae), a candidate for the biological control of *Prosopis* spp. (Fabaceae). *Biological Control* 13: 101-110.
- Dinerstein E, Wemmer CM. 1988. Fruits rhinoceros eat: dispersal of *Trewia nudiflora* in lowland Nepal. *Ecology* 69: 1768-1774.

- Edwards PB, Aschenborn HH. 1987. Patterns of nesting and dung burial in *Onitis* dung beetles: implications for pasture productivity and fly control. *Journal of Applied Ecology* 24: 837-852.
- Fuhlendorf SD, Smeins FE, Grant WE. 1996. Simulation of fire-sensitive ecological threshold: a case study of Ashe juniper on the Edwards Plateau of Texas, USA. *Ecological Modelling* 90: 245-255.
- Fuhlendorf SD. 1999. Ecological considerations for woody plant management. *Rangelands* 21: 12-15.
- Gardener CJ, McIvor JG, Jansen A. 1993. Passage of legume and grass seeds through the digestive tract of cattle and their survival in feces. *Journal of Applied Ecology* 30: 63-74.
- Gibbens RP, Beck RF, McNeely RP, Herbel CH. 1992. Recent rates of mesquite establishment in the northern Chihuahuan Desert. *Journal of Range Management* 45: 585-588.
- Gignoux J, Lahoreau G, Julliard R, Barot S. 2009. Establishment and early persistence of tree seedlings in an annually burned savanna. *Journal of Ecology* 97: 484-495.
- Goheen JR, Palmer TM, Keesing F, Riginos C, Young TP. 2010. Large herbivores facilitate savanna tree establishment via diverse and indirect pathways. *Journal of Animal Ecology* 79: 372-382.
- Grellier S, Barot S, Janeau J, Ward D. 2012. Grass competition is more important than seed ingestion by livestock for *Acacia* recruitment in South Africa. *Plant Ecology* 213: 899-908.

- Guillard P. 1967. Coprophagous beetles in pasture ecosystems. *Journal of the Australian Institute of Agricultural Science* 33: 30-34.
- Halevy G. 1974. Effects of gazelles and seed beetles (Bruchidae) on germination and establishment of *Acacia* species. *Israel Journal of Botany* 23: 120-126.
- Henery ML, Westoby M. 2001. Seed mass and seed nutrient content as predictors of seed output variations between species. *Oikos* 92: 479-490.
- Herrera CM, Jordano P, López-Soria L, Amat J. 1994. Recruitment of a mast-fruiting, bird dispersed tree: bridging frugivore activity and seedling establishment. *Ecological Monographs* 64: 315-344.
- Jeltsch F, Milton SJ, Dean van Rooyen N. 1998. Modelling the impact of small-scale heterogeneities on tree-grass coexistence in semi-arid savannas. *Journal of Ecology* 86: 780-793.
- Kraaij T, Ward D. 2006. Effects of rain, nitrogen, fire and grazing on tree recruitment and early survival in bush-encroached savanna, South Africa. *Plant Ecology* 186: 235-246.
- Leishman MR, Wright IJ, Moles AT, Westoby M. 2000. The evolutionary ecology of seed size. In: Fenner M (ed), *Seeds – The Ecology of Regeneration in Plant Communities*. CAB International, Wallingford, UK. pp 31-57.
- Lerner P, Peinetti R. 1996. Importance of predation and germination on losses from the seed bank of calden (*Prosopis caldenia*). *Journal of Range Management* 49: 147-150.

- Little TM, Hills FJ. 1972. *Statistical Methods in Agricultural Research*. University of California, Davis, CA, USA. pp 93-101.
- Meyer SE, Pendleton BK. 2005. Factors affecting seed germination and seedling establishment of a long-lived desert shrub (*Coleogyne ramosissima*: Roasaceae). *Plant Ecology* 178: 171-187.
- Miller MF, Coe M. 1993. Is it advantageous for *Acacia* seeds to be eaten by ungulates? *Oikos* 66: 346-368.
- Miller MF. 1995. *Acacia* seed survival, seed germination and seedling growth following pod consumption by large herbivores and seed chewing rodents. *African Journal of Ecology* 33:194-210.
- Moles AT, Ackerly DD, Webb CO, Tweddle JC, Dickie JB, Westoby M. 2005. A brief history of seed size. *Science* 307: 576-580.
- Mucina L, Rutherford MC. 2006. *The Vegetation of South Africa, Lesotho and Swaziland*. Strelitzia 19, South African National Biodiversity Institute, Pretoria, South Africa.
- Mwalyosi RBB. 1990. The dynamic ecology of *Acacia tortilis* woodland in Lake Manyara National Park, Tanzania. *African Journal of Ecology* 28: 189-199.
- Nathan R, Muller-Landau HC. 2000. Spatial pattern of seed dispersal, their determinants and consequences of recruitment. *Trends in Ecology and Evolution* 15: 278-285.
- O'Connor TG. 1995. *Acacia karroo* invasion of grassland: environmental and biotic effects influencing seedling emergence and establishment. *Oecologia* 103: 214-223.

- O'Connor TG. 1996. Individual, population and community response of woody plants to browsing in African savannas. *Bulletin of the Grassland Society of southern Africa, Supplement 7*: 14-18.
- Or K, Ward D. 2003. Three-way interaction between *Acacia*, large mammalian herbivores and bruchid beetles- a review. *African Journal of Ecology* 41: 257-265.
- Or K, Ward D. 2007. Maternal effects on the life histories of bruchid beetles infesting *Acacia raddiana* in the Negev desert, Israel. *Entomologia Experimentalis et Applicata* 122: 165-170.
- Pakeman RJ, Small JL. 2009. Potential and realised contribution of endozoochory to seedling establishment. *Basic and Applied Ecology* 10: 656-661.
- Robbins CT. 1993. *Wildlife Feeding and Nutrition* (2<sup>nd</sup> edn). Academic Press, New York.
- Rohner C, Ward D. 1999. Large mammalian herbivores and the conservation of arid *Acacia* stands in the Middle East. *Conservation Biology* 13: 1162-1171.
- Sankaran M, Ratnam J, Hanan NP. 2004. Tree-grass coexistence in savannas revisited – insights from an examination of assumptions and mechanisms invoked in existing models. *Ecology Letters* 7: 480-490.
- SAS Institute. 2002. *SAS/STAT User's Guide*, Version 9.2. SAS Institute, Cary, NC, USA.
- Schupp EW, Fuentes M. 1995. Spatial patterns of seed dispersal and the unification of plant population ecology. *Ecoscience* 2: 267-275.



- Schupp EW, Gomes JM, Jimenez JE, Fuentes M. 1997. Dispersal of *Juniperus occidentalis* (Western Juniper) seeds by frugivorous mammals on Juniper Mountain, southeastern Oregon. *Great Basin Naturalist* 57: 74-78.
- Scholes RJ, Archer SR. 1997. Tree-grass interactions in savannas. *Annual Review of Ecology and Systematics* 28: 517-544.
- Shapiro SS, Wilks MB. 1965. An analysis of variance test for normality (complete samples). *Biometrika* 52: 591-611.
- Shayo CM, Uden P. 1998. Recovery of seed of four African browse shrubs ingested by cattle, sheep and goats and the effect of ingestion, hot water and acid treatment on the viability of the seeds. *Tropical Grasslands* 32: 195-200.
- Simao Neto M, Jones RM, Ratcliff D. 1987. Recovery of pasture seed ingested by ruminants. 1. Seed of six tropical pasture species fed to cattle, sheep and goats. *Australian Journal of Experimental Agriculture* 27: 239-246.
- Smit GN. 2004. An approach to tree thinning to structure southern African savannas for long-term restoration from bush encroachment. *Journal of Environmental Management* 71: 179-191.
- Snedecor GW, Cochran WG. 1967. *Statistical Methods* (6th edn). Iowa State University Press, Ames, IA, USA.
- Tanner JC, Reed JD, Owen E. 1990. The nutritive value of fruits (pods with seeds) from four *Acacia* species compared with extracted noug (*Goizotia abyssica*) meal as animal

- supplements to maize stover for Ethiopian highland sheep. *Animal Production* 51: 127-133.
- Tjelele TJ, Dziba LE, Pule HT. 2012. Recovery and germination of *Dichrostachys cinerea* seeds fed to goats (*Capra hircus*). *Rangeland Ecology and Management* 65: 105-108.
- Traveset A, Bermejo T, Willson M. 2001. Effect of manure composition on seedling emergence and growth of two common shrub species of southeast Alaska. *Plant Ecology* 155: 29-34.
- Trollope WSW. 1980. Controlling bush encroachment with fire in the savanna areas of South Africa. *Proceedings of the Grassland Society of southern Africa* 15: 173-177.
- Turnbull LA, Rees M, Crawley MJ. 1999. Seed mass and the competition/colonization trade-off: a sowing experiment. *Oikos* 88: 899-912.
- Van Wyk B, Van Vyk P. 2007. *Trees of southern Africa*. Struik, Cape Town, South Africa.
- Ward D. 2005. Do we understand the causes of bush encroachment in African savannas? *African Journal of Range and Forage Science* 22: 101-105.
- Weitbrecht K, Müller K, Leubner-Metzger G. 2011. First off the mark: early seed germination. *Journal of Experimental Botany* 62: 3289-3309.
- Westoby M, Leishman M, Lord J. 1996. Comparative ecology of seed size and dispersal. *Philosophical Transactions of the Royal Society of London, Series B* 351: 1309-1318.
- Whitacre MK, Call CA. 2006. Recovery and germinability of native seed fed to cattle. *Western North American Naturalist* 66: 121-128.

Wiegand K, Saltz D, Ward D. 2006. A patch-dynamics approach to savanna dynamics and woody plant encroachment – insights from an arid savanna. *Perspectives in Plant Ecology, Evolution and Systematics* 7: 229-242.

Wilson TB, ETF Witkowski 1998. Water requirements for germination and early seedling establishment in four African savanna woody plant species. *Journal of Arid Environments* 38: 541-550.

**Table 1:** *F* values and *P* values for the effects of the five treatments and their interactions on seed germination, seedling survival and seedling recruitment.

Treatments	Seed germination			Seedling survival		Seedling recruitment	
	df	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Animal species	1	838.9	<0.0001	645.8	<0.0001	775.7	<0.0001
Seed recovery day	1	758.0	<0.0001	719.6	<0.0001	671.4	<0.0001
Seed species	1	500.4	<0.0001	547.7	<0.0001	489.6	<0.0001
Germination treatment	2	25.8	<0.0001	32.6	<0.0001	35.4	<0.0001
Season	1	14.2	0.0002	29.0	<0.0001	9.6	0.0020
Animal spp.* seed spp.	2	65.8	<0.0001	39.5	<0.0001	54.8	<0.0001
Animal spp.* seed recovery day	2	796.9	<0.0001	636.7	<0.0001	703.1	<0.0001
Seed recovery day* germination treatment	2	12.2	<0.0001	17.1	<0.0001	6.7	<0.0001
Animal spp.* day* germination treatment	4	10.58	<0.0001	10.23	0.0007	5.5	0.0003

#### 4.9. Figure Legends

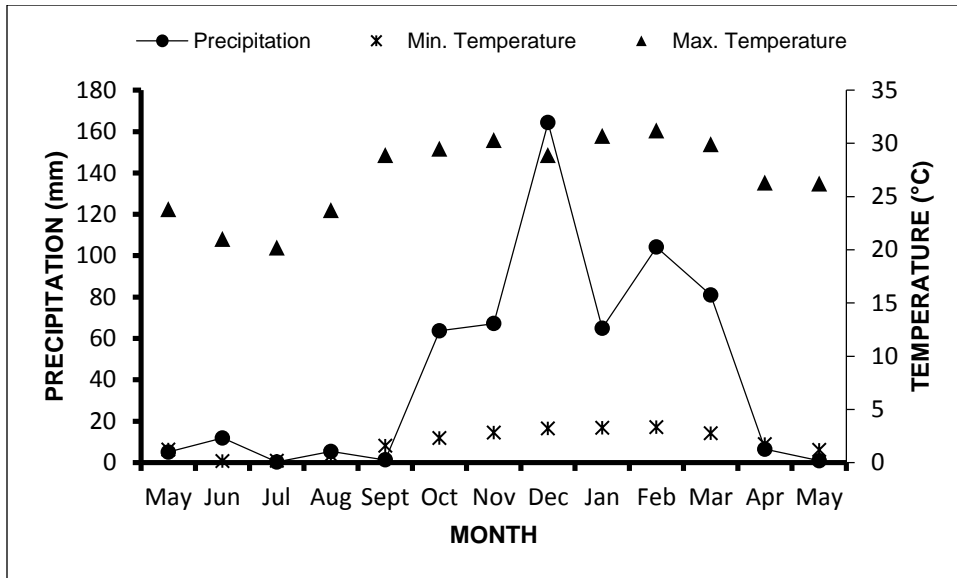
**Figure 1:** Mean monthly precipitation and minimum and maximum temperatures for 2011-2012 at the Agricultural Research Council's Roodeplaat Experimental Farm.

**Figure 2:** Significant interaction effect of animal species (cattle and goats), seed species (*D. cinerea* and *A. nilotica*) and seed recovery day on mean cumulative percentage seed recovery. Error bars represent standard errors (SE). Fisher's *post hoc* test was used.

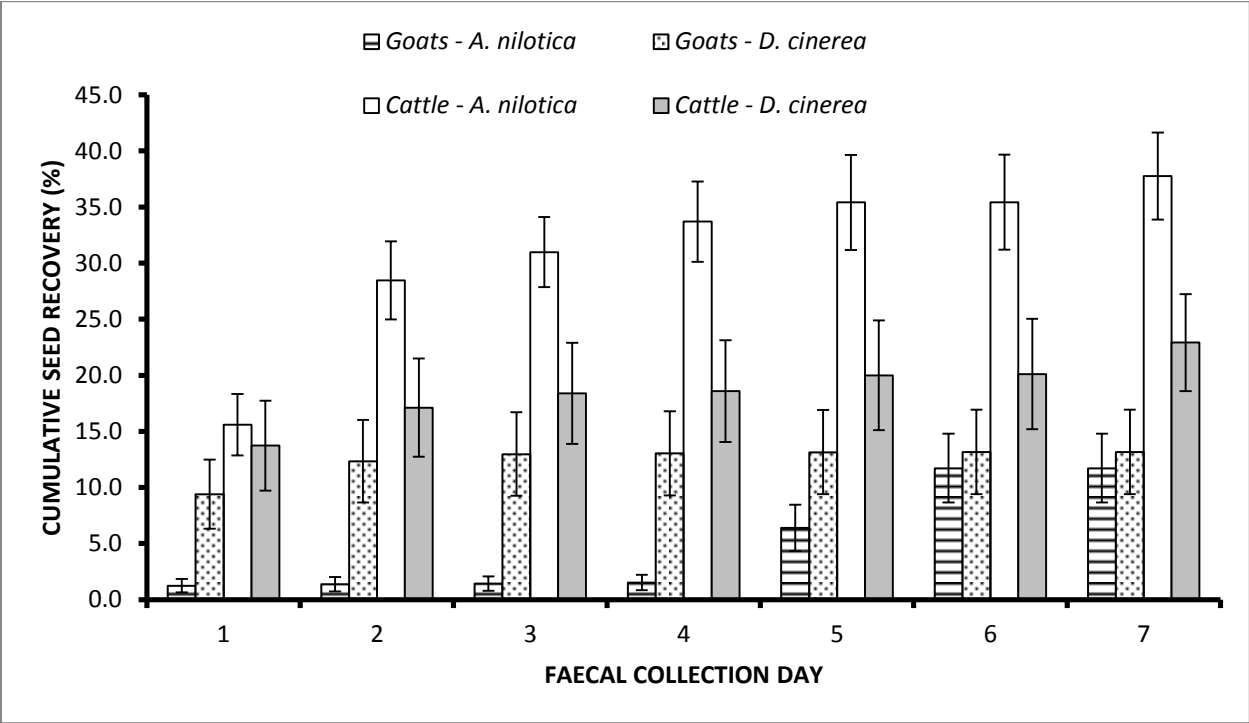
**Figure 3:** The interaction effect on seedling emergence of animal species (goats, cattle) and unpassed/untreated seeds (i.e. not ingested), seed recovery day (first three days (F3)), last four days (L4)) and planting methods (seeds placed on top of the soil with no dung (Top), seeds buried 2 cm in the soil with no dung (ND), seeds buried 2 cm in the soil with dung (WD). Bars represent standard errors (S.E). Fisher's *post hoc* test was used.

**Figure 4:** The interaction effect on seedling survival of animal species (goats, cattle) and unpassed/untreated seeds (i.e. not ingested), seed recovery day (first three days (F3)), last four days (L4)) and planting methods (seeds placed on top of the soil with no dung (Top), seeds buried 2 cm in the soil with no dung (ND), seeds buried 2 cm in the soil with dung (WD). Bars represent standard errors (S.E). Fisher's *post hoc* test was used.

**Figure 5:** The interaction effect of animal species (goats, cattle) and unpassed/untreated seeds (i.e. not ingested), seed recovery day (first three days (F3)), last four days (L4)) and planting methods (seeds placed on top of the soil with no dung (Top), seeds buried 2 cm in the soil with no dung (ND), seeds buried 2 cm in the soil with dung (WD) on seedling recruitment. Bars represent standard errors (S.E). Fisher's *post hoc* test was used.

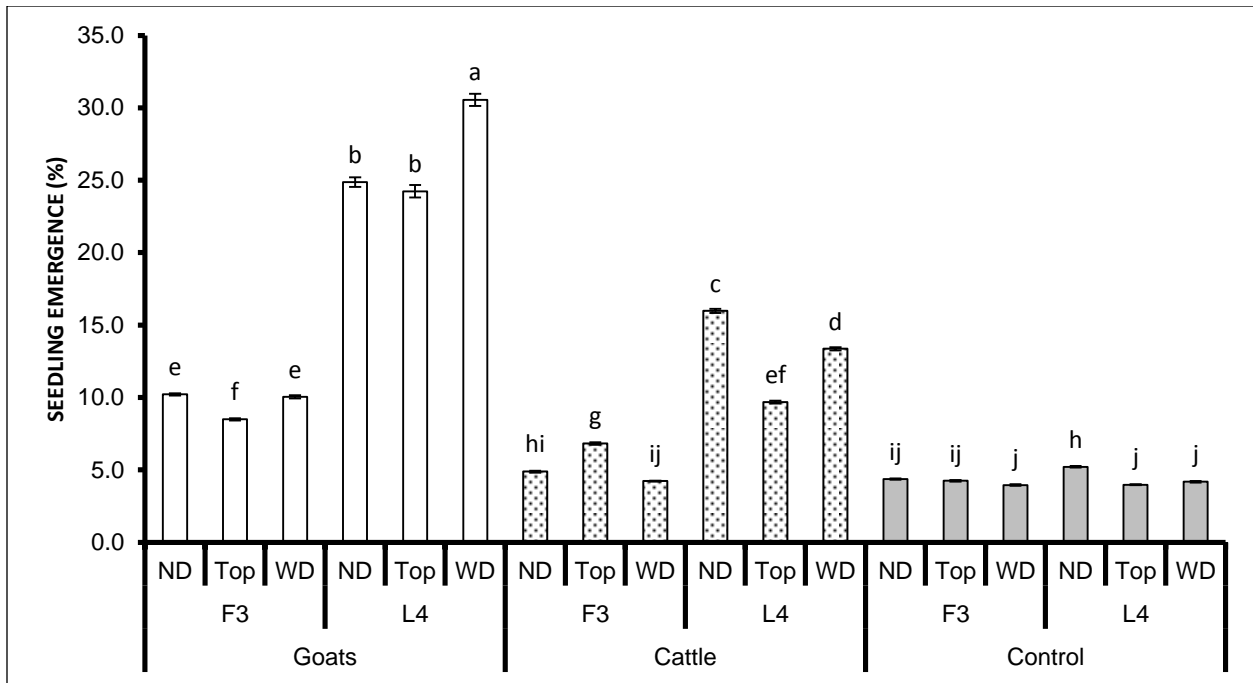


**Figure 1:**



**Figure 2:**





**Figure 3:**

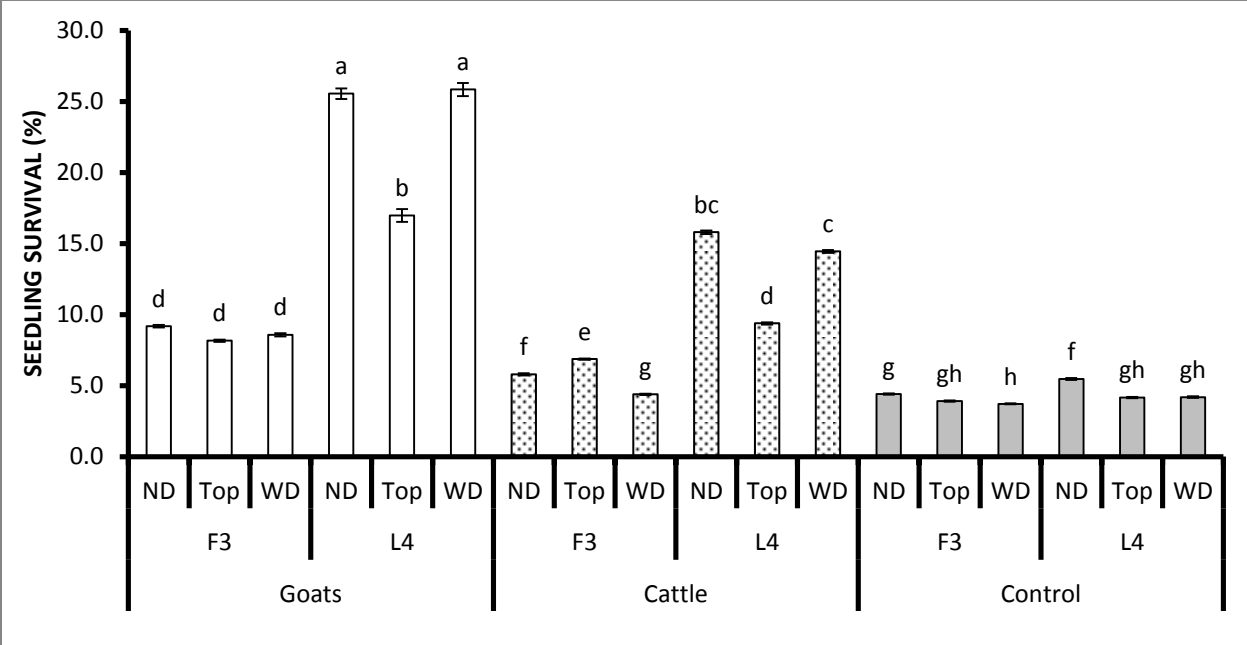
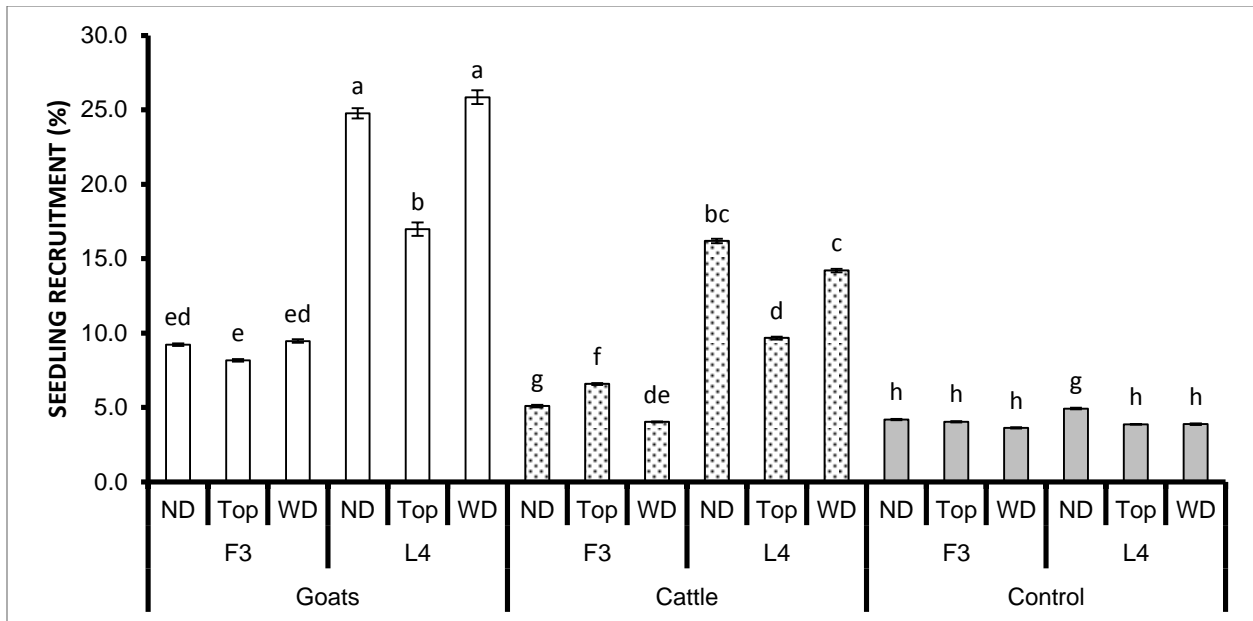


Figure 4:



**Figure 5:**

## Chapter 5

### **The effects of seed ingestion by livestock, dung fertilization, trampling, grass competition and fire on seedling establishment of two woody plant species**

Julius Tjelele<sup>ab\*</sup>, David Ward<sup>b</sup> and Luthando Dziba<sup>c</sup>

<sup>a</sup>Agricultural Research Council, Animal Production Institute, Irene, 0062, South Africa, e-mail:

[jtjelele@arc.agric.za](mailto:jtjelele@arc.agric.za); <sup>b</sup>School of Life Sciences, College of Agriculture, Engineering and Science,

University of KwaZulu-Natal, Scottsville 3209, South Africa, e-mail:

[davidmercerward@gmail.com](mailto:davidmercerward@gmail.com); <sup>c</sup>CSIR: Natural Resources and the Environment, Pretoria 0001,

South Africa, e-mail: [ldziba@csir.co.za](mailto:ldziba@csir.co.za)

Correspondence author: Julius Tjelele, Agricultural Research Council, Animal Production

Institute, Private Bag X2, Irene, 0062, South Africa. Email: [jtjelele@arc.agric.za](mailto:jtjelele@arc.agric.za), Tel: (+27) 12

672 9314, Fax: (+27) 12 672 9314

**Formatted for submission to *Journal of Applied Ecology***

## 5.1. Summary

1. The increasing rate of woody plant encroachment in grasslands or savannas remains a challenge to livestock farmers. The causes and control measures of woody plant encroachment are of common interest, especially where it negatively affects the objectives of an agricultural enterprise.
2. The objectives of this study were to determine the effects of gut passage (goats, cattle), dung (nutrients), fire, grass competition and trampling on establishment of *A. nilotica* and *D. cinerea* seedlings.
3. Germination trials were subjected to the following treatments: 1) seed passage through the gut of cattle and goats and unpassed/ untreated seeds (i.e. not ingested), 2) dung and control (no dung), 3) grass and control (mowed grass), 4) fire and control (no fire), 5) trampling and control (no trampling).
4. The interaction of animal species, grass and fire had an effect on seedling recruitment ( $P < 0.0052$ ). Seeds retrieved from goats and planted with no grass and with fire ( $6.81\% \pm 0.33$ ) had a significant effect on seedling recruitment than seeds retrieved from goats and planted with grass and no fire ( $2.98\% \pm 0.33$ ). Significantly more *D. cinerea* and *A. nilotica* seeds germinated following seed ingestion by goats ( $3.59\% \pm 0.16$ ) than cattle ( $1.93\% \pm 0.09$ ) and control or untreated seeds ( $1.69\% \pm 0.11$ ).
5. *Synthesis and applications.* Less dense grass cover, which resulted in reduced grass competition with tree seedlings for light, space and water, and improved seed scarification due to gut passage were vital for emergence and recruitment of *Acacia* seedlings. These results will

contribute considerably to the understanding of the recruitment phase of woody plant encroachment.

**Key words:** dung nutrients, seed scarification, survival, tree-grass seedling competition, woody plant encroachment

## **5.2. Introduction**

An increase in woody vegetation density (Tews, Moloney & Jeltsch 2004; Ward 2005; van Auken 2009), known as woody plant encroachment, has been widely reported in southern Africa (Kraaij & Ward 2006; Wigley, Bond & Hoffman 2009). The increasing rate of woody plant encroachment in grassland and/or savanna is a challenge to livestock farmers (Kraaij & Ward 2006; Wigley, Bond & Hoffman 2010; Kgosikoma, Harvie & Mojeremane 2012). An increase in woody plant density suppresses the productivity of herbaceous plant species (Hagos & Smit 2005; Ward 2005) and eventually reduces rangeland carrying capacity (Ward 2005; Wiegand, Saltz & Ward 2006). The reduction in carrying capacity is of great concern because African savannas have a rapidly growing human population (Lamprey 1983; Ward 2005). Despite the widespread occurrence of woody plant encroachment (Hoffman & Ashwell 2001), its dynamics are not entirely understood, particularly processes that lead to woody plant encroachment and dominance (Scholes & Archer 1997; Higgins, Bond & Trollope 2000; Ward 2005; Wiegand, Ward & Saltz 2005; Sharam, Sinclair & Turkington 2006). Consequently, there is considerable interest in understanding woody plant encroachment (Ward 2005) in order to improve livestock production.

There is agreement that rainfall, soil, nutrients, herbivory and fire are key variables affecting tree-grass ratios (Sankaran, Ratman & Hanan 2004; Ward 2005; Kraaij & Ward 2006). However, little attention has been paid to the influence of seed recovery by herbivores on woody plant encroachment (Or & Ward 2007). Grazing herbivores reduce the ability of grasses to compete with tree seedlings for resources through grazing (Jeltsch *et al.* 1996; Kraaij & Ward 2006; Goheen *et al.* 2010). Conversely, herbivores disperse viable woody plant seeds (Tjelele, Dziba & Pule 2012), and may increase seedling emergence, seedling survival and recruitment of woody plant species through gut passage and dung fertilization (Miller & Coe 1993; Or & Ward 2003; Bodmer & Ward 2006; Grellier *et al.* 2012; see also Chapter 4).

Pods of certain woody plant species form an important part of the diet of goats, and to a certain extent sheep and cattle, and satisfy their nutritional requirements during the dry season (Janzen 1984; Rohner & Ward 1999). *Acacia* and *Dichrostachys* seeds have hard seed coats, which enable some of the ingested seeds to be passed out unharmed in the faeces (Or & Ward 2003). Herbivores with large body size such as cattle have longer retention time than goats (Bodmer & Ward 2006). A positive correlation between germination rates and herbivore size has been found (Bodmer & Ward 2006; Rodrigues-Pérez, Wiegand & Ward 2011). Furthermore, seed recovery and the distance that seeds may be dispersed depends positively on herbivore retention time and body size (Or & Ward 2003; Bodmer & Ward 2006). However, other factors such as diet quality and seed characteristics (size, hardness and shape) may have different effects on germination (see Chapter 3; Gardener, McIvor & Jansen 1993; Schupp *et al.* 1997; Whitacre & Call 2006).

Seed size and seed mass are important traits in the seedling establishment of a plant species (Moles & Westoby 2002; Moles *et al.* 2005). The number of seeds a plant can produce is

related to the mass of the seed produced (Smith & Fretwell 1974; Jakobson & Eriksson 2000; Henery & Westoby 2002). The greater seed output of small-seeded species such as *D. cinerea* may reach a greater proportion of potential establishment sites than large-seeded species e.g. *A. nilotica* (Moles & Westoby 2002). The lower output of large-seeded species may be compensated during seedlings establishment, as seedlings from large seeds are generally better at tolerating stress such as drought, defoliation, shade and plant competition (Leishman *et al.* 2000). Large-seeded species would be expected to show a greater seedling recruitment (this is the time when the reserves are deployed from the cotyledons) than small-seeded species (Westoby, Leishman & Lord 1996; Turnbull, Rees & Crawley 1999).

Herbivores have a considerable impact on savanna structure because they trample, urinate and defaecate (Skarpe 1991; Danell *et al.* 2003). Furthermore, intense grazing and trampling may result in low plant cover, low or high soil nutrients (depending on whether dung is involved), low water infiltration, reduced water availability and high erosion (McNaughton 1983), especially on a clay wet soil (pers. obs.). Trampling may therefore reduce tree seedling survival, establishment and recruitment due to both the inability of seedling roots to penetrate the soil and high water run-off and reduced water availability for seedlings (Valentin & Bresson 1992).

Multi-factorial experiments have been emphasized as one of the possible ways to understand the causes and develop management strategies of woody plant encroachment (Ward 2005; Kraaij & Ward 2006; James, Svejcar & Rinella 2011). Seed recovery, seedling emergence, seedling establishment and recruitment are crucial processes (early stages of the life cycle) in plant population dynamics because they usually influence the distribution and abundance of plant species (Seabloom *et al.* 2003; James, Svejcar & Rinella 2011). The appearance of a radicle marks the end of seed germination (the emergence and development from the seed embryo) and



the beginning of seedling establishment, a period that ends when the seedling has exhausted the food reserves stored in the seed (Westoby *et al.* 1996; Weitbrecht *et al.* 2011). Seedling recruitment refers to the process by which new individuals/seedlings establish a new population or are added to an existing population (Herrera *et al.* 1994; Schupp & Fuentes 1995). Environmental factors such as water stress, light availability and leaf litter cover could constrain the emergence, establishment and recruitment of tree seedlings (Meyer & Pendleton 2005). This may suggest that seeds recovered from herbivores are not necessarily guaranteed to germinate. Additionally, seedling establishment and recruitment, which are pre-requisites for maintaining or increasing tree abundance and are influential in woody plant encroachment, are not assured (Nathan & Muller-Landau 2000; Kraaij & Ward 2006).

Overall, there is unlikely to be a single cause of woody plant encroachment, but rather a combination of interacting factors (van Auken 2009; Grellier *et al.* 2012). To better understand the mechanisms involved, we studied the effects of gut passage (goats and cattle), dung (nutrients), grass competition, fire and trampling on seedling emergence, seedling survival and recruitment of *Dichrostachys cinerea* and *Acacia nilotica* seeds.

### **5.3. Materials and methods**

#### **5.3.1. STUDY AREA**

The study was done at the Agricultural Research Council's Roodeplaat Experimental Farm, Gauteng province, South Africa (28°19'E, 25°35'S). The natural vegetation component of the farm used for livestock production and game makes up an area of approximately 2100 ha. The

vegetation type of Roodeplaats is classified as Marikana Thornveld by Mucina & Rutherford (2006), and is generally characterized by open *Acacia karroo* and *A. caffra* woodlands in the valleys (Mucina & Rutherford 2006). The main grass species on the site were *Digitaria eriantha* and *Pennisetum clandestinum*. The general soil type is Hutton. The mean annual rainfall is 646 mm, and the minimum and maximum summer and winter temperatures are 20–29 °C and 2–16 °C.

### 5.3.2. FIELD GERMINATION

The experimental design consisted of 1 × 1 m plots, with 50 seeds per plot placed at 1 cm depth in the soil (following Brown & Booysen 1967). All plots were separated by a 1 m buffer zone. The seedling emergence trial consisted of the following completely randomized design with three replicates per treatment: 1) passage through goats or cattle or unpassed/untreated seeds (i.e. not ingested), 2) dung and control (no dung), 3) grass competition and mowed grass, 4) fire and control (no fire), and 5) trampling and control (no trampling) (Table 1).

Fifty *D. cinerea* seeds and 50 *A. nilotica* seeds retrieved from goats and unpassed/untreated seeds were planted per plot (i.e. 50 *D. cinerea*, 50 *A. nilotica* and 50 unpassed/untreated seeds of each species were planted in separate plots) in three replicates with 16 combinations (96 plots and 4800 seeds). Another 50 *D. cinerea* seeds and 50 *A. nilotica* seeds retrieved from cattle and untreated seeds were planted per plot in three replicates with 16 combinations (96 plots and 4800 seeds). In total, 9600 seeds were planted in 192 plots for goats and 192 plots for cattle. The same was true for the controls.

#### *5.3.2.1. Goats, cattle and unpassed/untreated seeds*

Twenty Bonsmara cows and 20 indigenous female goats were divided into two groups of 10 per animal species. One cow from each group of 10 cows was fed either 1500 *D. cinerea* seeds or *A. nilotica* seeds. One goat from each group of 10 goats was also fed 1500 *D. cinerea* or *A. nilotica* seeds. Retrieved undamaged seeds (following techniques outlined by Tjelele, Pule & Dziba 2012; see also Chapter 2) were used during the field seedling emergence trial.

#### *5.3.2.2. Grass competition*

The mowed-grass treatments were manually cut to ground height before planting and thereafter every month for the duration of the experiment to either simulate grass competition with tree seedlings the indirect effect of grazing (reduced grass competition). Precautions were taken to conserve tree seedlings when cutting grasses around them.

#### *5.3.2.3. Dung fertilization*

Dung provides a suitable nutritive medium for seedling emergence and establishment (Coughenour & Detling 1986; Dinerstein & Wemmer 1988). Dung collected from experimental animals (goats and cow) after the seed recovery trial (i.e. when there were no seeds in the dung) was used for planting to simulate the effect of dung as nutrient input. About 60 cm<sup>3</sup> of homogenized fresh dung was applied to each seed in dung plots just after planting (see Grellier et al. 2012).

#### 5.3.2.4. *Trampling*

Twenty cattle were corralled for 15 days at the trampling treatment site, prior to planting. A goat trampling treatment was not done because their effects are considered to be negligible (pers. obs.).

#### 5.3.2.5. *Fire*

Grasses are superior competitors to tree seedlings for resources such as water, light, space and nutrients (Wilson 1988; Kanz 2001; Ward & Esler 2010). The fire treatment was applied using old dry grass bales prior to seed planting. Two dry bales were evenly spread on fire plots to ensure that all (above-ground) grasses were burnt.

### 5.4.3. MONITORING OF FIELD SEEDLING EMERGENCE TRIAL

The data collection lasted for nine months from May 2012 to January 2013. Seedling emergence and establishment were monitored over three seasons (winter and summer). The austral winter is the time during which animals consume and distribute seeds of different woody plant species (*D. cinerea* and *A. nilotica* seeds are produced in April) and summer to capture emergence and establishment during the rainy season. Seeds were monitored monthly to record germinated seeds and seedling survival. Seedling recruitment was determined from the difference between the seeds emerged and seedlings that had died since the beginning of the experiment (see also Grellier *et al.* 2012).

#### 5.4.4. STATISTICAL ANALYSIS

The germination experiment was subjected to a ( $3 \times 2^5$ ) factorial analysis of variance (ANOVA) with the following main factors: seeds retrieved from two animal species ((goats, cattle) and unpassed/untreated seeds (i.e. not ingested by goats or cattle)), two seed species (*A. nilotica*, *D. cinerea*), two dung treatment levels (dung and no dung), two fire treatment levels (fire and no fire), two trampling treatment levels (trampling and no trampling) and two grass treatment (grass and mowed grass). The trampling treatment was only applied for cattle. We analysed the effects of the six treatments and their interactions on seed germination, seedling survival and seedling recruitment after a logit transformation (Snedecor & Cochran 1967) with normal residuals and homogeneity of variance. Repeated measurements were included in the analysis as a sub-plot factor (Little & Hills 1972). The standardized residuals were tested for normality using a Shapiro-Wilks test (1965). Differences between means were considered significant at 5% level using Student's t-LSD (Least significant difference). The analysis was done using SAS statistical software (SAS Institute 2002) for a completely randomized design.

### 5.4. Results

#### 5.4.1. DRY SEASON (WINTER)

The interaction of animal species, grass and fire had a significant effect on seedling emergence ( $P < 0.0167$ ), seedling survival ( $P < 0.0317$ ) and seedling recruitment ( $P < 0.0052$ ; Table 2). Seedling emergence was significantly affected by passage through the gut of goats, mowed grass (Gc), and fire (F) ( $8.68\% \pm 0.36$ ) and goats, grass, and fire ( $4.47\% \pm 0.24$ ). However, seedling emergence was not significantly affected by goats, mowed grass (Gc), and no fire (Fc) ( $1.32\% \pm 0.22$ ) and seeds retrieved from goats, grass (G), and no fire (Fc) ( $1.08\% \pm 0.09$ ). The same

pattern was observed in seedling survival. The treatment combination of goats, mowed grass (Gc), and fire (F) ( $6.81\% \pm 0.33$ ) and goats, grass (G), and fire (F) ( $2.98\% \pm 0.33$ ) significantly affected seedling recruitment (Figure 1).

During the dry season, seedling emergence ( $P < 0.0136$ ), seedling survival ( $P < 0.0227$ ) and seedling recruitment ( $P < 0.0312$ ) were significantly affected by animal species, dung and grass (Table 2). Seeds retrieved from goats, planted with dung (D), and mowed grass (Gc) had significantly better emergence ( $4.71\% \pm 0.43$ ) than goat, dung (D), and grass (G) ( $1.78\% \pm 0.27$ ). The same was true for seedling survival and seedling recruitment (see Figure 2). Surprisingly, there was no significant difference in seedling emergence, seedling survival and seedling recruitment from seeds retrieved from cattle and unpassed/ untreated seeds, planted with dung (D) or no dung (Dc) and grass (G) or mowed grass (Gc) (Figure 2).

The interaction effect of animal species, seed species, dung and fire had a significant effect on seedling emergence ( $P < 0.0236$ ; Table 2). The treatment combination of goats, *A. nilotica* seeds, no dung (Dc), fire (F) ( $13.96\% \pm 0.33$ ) and goats, *A. nilotica* seeds, dung (D), and fire (F) ( $10.53\% \pm 0.42$ ) emerged significantly more than goats, *A. nilotica* seeds, no dung (Dc), and no fire (Fc) ( $1.18\% \pm 0.19$ ) and goats, *A. nilotica* seeds, dung (D), and no fire (Fc) ( $1.48\% \pm 0.42$ ). There was a significant difference in *D. cinerea* seedling emergence between those ingested by goats, with dung (D), and with fire (F) ( $4.39\% \pm 0.50$ ) and seeds ingested by goats, no dung (Dc), and with fire (F) ( $1.71\% \pm 0.39$ ).

There was a significant interaction of animal species, seed species, grass and fire on seedling emergence ( $P < 0.0279$ ; Table 2). During the dry season, *A. nilotica* seedling emergence was significantly higher after goat ingestion, with grass (G), and with fire (F) ( $9.30\% \pm 0.21$ )

than after goat ingestion, with grass (G), and no fire (Fc) ( $1.42\% \pm 0.24$ ). During the dry season, *D. cinerea* seedling emergence was significantly higher after goat ingestion, with mowed grass (Gc), and fire (F) ( $7.62\% \pm 0.33$ ) than after goat ingestion, with mowed grass (Gc), and no fire (Fc) ( $3.12\% \pm 0.60$ ; Figure 3). Emergence of unpassed/untreated *A. nilotica* seedling with mowed grass (Gc), and fire (F) ( $3.88\% \pm 0.31$ ) was significantly greater than unpassed with mowed grass (Gc), and no fire (Fc) ( $0.98\% \pm 0.00$ ; Figure 3).

The interaction of seed species, dung and fire had a significant effect on seedling survival ( $P < 0.0092$ ; Table 2). *A. nilotica* seeds, no dung (Dc), fire (F) ( $5.14\% \pm 0.23$ ) and *D. cinerea* seeds, dung (D), fire (F) ( $4.35\% \pm 0.21$ ) survived significantly better than *A. nilotica* seeds, no dung (Dc), no fire (Fc) ( $1.59\% \pm 0.15$ ) and *D. cinerea* seeds, dung (D), no fire (Fc) ( $1.75\% \pm 0.17$ ; Figure 4).

Seeds retrieved from goats ( $3.59\% \pm 0.16$ ) recruited significantly better than seeds retrieved from cattle ( $1.93\% \pm 0.09$ ) and control/untreated seeds ( $1.69\% \pm 0.11$ ). Significantly more *A. nilotica* seeds ( $1.73\% \pm 0.07$ ) recruited than *D. cinerea* seeds ( $1.62\% \pm 0.06$ ). The fire ( $2.95 \pm 0.11$ ) treatment (which was used to remove the above-ground grass) had significantly more seedling recruitment than no fire ( $1.61 \pm 0.09$ ) treatment.

#### 5.4.2. WET SEASON (SUMMER)

The interaction of seed species, dung and grass had a significant effect on seedling emergence ( $P < 0.0099$ ), seedling establishment ( $P < 0.0305$ ) and seedling recruitment ( $P < 0.0036$ ; Table 2). The seedling emergence of *A. nilotica* with no dung (Dc), and mowed grass (Gc) ( $3.75\% \pm 0.19$ ) was significantly greater than with no dung (Dc), and grass (G) ( $1.88\% \pm 0.17$ ). Seedling

emergence of *D. cinerea* with dung (D), and mowed grass (Gc) ( $3.15\% \pm 0.23$ ) was significantly greater than *D. cinerea* with dung (D) and grass (G) ( $2.00\% \pm 0.21$ ). Seedling emergence of *A. nilotica* with no dung (Dc) and mowed grass (Gc) ( $3.75\% \pm 0.19$ ) was significantly greater than (Figure 5).

## 5.5. Discussion

### 5.5.1. SEEDLING EMERGENCE, SEEDLING SURVIVAL AND RECRUITMENT

The results of this study showed that goat-ingested seeds (3.94%) had significantly higher germination percentage than cattle-ingested seeds (1.97%) and control/untreated (1.74%) (i.e. no passage through the gut of livestock). Bodmer & Ward (2006) have shown that seed germination increases with animal body size. The opposite results were found in this study. However, these results are consistent with those of Grellier *et al.* (2012) in KwaZulu-Natal, South Africa. Even though cattle and untreated seeds were not significantly different in seedling emergence and seedling recruitment, it is clear that acid scarification in the gut of herbivores usually favours seedling emergence (Bodmer & Ward 2006; Maclean *et al.* 2011; Grellier *et al.* 2012; Tjelele, Pule & Dziba 2012). Longer retention of seeds ingested by large bodied-animals, e.g. cattle, may result in substantial damage to the seeds (Brown & Archer 1987; Miller 1995; Rohner & Ward 1999), which may cause significantly lower seedling emergence after cattle ingestion than from goats. Recovery of viable seeds depends on the interaction of different factors such as seed characteristics (hardness, shape), associated diet and animal species (see Chapter 3), which may result in increased or decreased germination. For instance, in this study, there were relatively



more damaged seeds observed from cattle ingestion than goat ingestion (pers. obs.). The large *A. nilotica* seeds recruited significantly better than the smaller *D. cinerea* seeds. These results are consistent with those of Leishman *et al.* (2000) who reported seedlings from large-seeded species are expected to show a greater seedling recruitment than small-seeded species.

Seedling emergence, seedling survival and recruitment increased significantly with fire, grass mowing and seed passage through the gut of goats but not with dung fertilization. Dung was expected to provide a suitable nutritive medium for seed germination and seedling survival (Coughenour & Detling 1986; Dinerstein & Wemmer 1988). The opposite results were found in this study, which is consistent with the results of Grellier *et al.* (2012). The non-significant seedling emergence and seedling survival with addition of dung may have been caused by dry dung because of the creation of a hard dung layer (Coe & Coe 1987; Grellier *et al.* 2012). In addition, dung may not have been decomposed and nutrients incorporated into the soil (Brown *et al.* 2010), which resulted in a negative effect of dung on seedling emergence. Another possible reason might be competition with other plant species (Traveset, Bermejo & Willson 2001).

The most interesting results were the positive effect of fire treatments on seedling emergence and seedling recruitment in this study. Grasses are superior competitors to tree seedlings for resources such as water, light, space and nutrients (Wilson 1988; Coffin & Lauenroth 1990; Bush & Van Auken 1995; Jeltsch *et al.* 1996; Kanz 2001; Ward & Esler 2010). For this reason, *A. nilotica* and *D. cinerea* seedlings and other woody plant species may be disadvantaged by germinating and surviving among grasses without fire (Cramer *et al.* 2007). The use of fire to remove the above-ground grass had the most important effect on seedling emergence and seedling recruitment, most probably because of less dense grass cover and therefore reduced competition between tree seedlings and grasses (O'Connor 1995; Campbell &

Clarke 2006). It is clear from this study that combinations of interacting factors (e.g. animal species, seed species, grass competition, dung fertilization and fire) influence seedling recruitment of *D. cinerea* and *A. nilotica* seeds. Regardless of whether there was fire or not, dung or not, the effect of livestock (especially goats) played an important role in seed scarification. Furthermore, livestock may also disperse viable seeds away from the mother tree, which may favour competition with adult trees (Kambatuku *et al.* 2011).

Seedling emergence and survival of seedlings throughout the dry season (when water is scarce) plays a crucial role in plant population dynamics. Rainfall plays an important role in germination, seedling survival and recruitment (O'Connor 1995; Wilson & Witkowski 1998). The effect of fire treatment, which removed grass competition, positively affected survival and recruitment of *D. cinerea* and *A. nilotica* seedlings (Rohner & Ward 1999; Bush & Van Auken 1995; Jeltsch *et al.* 1996, Grellier *et al.* 2012). In addition, soil moisture from precipitation (Schwinning *et al.* 2002) is the most limiting factor in the growth of tree and grass seedlings. Grasses are able to take up and use rainwater at a much faster rate than woody trees (Walter 1939; Frost *et al.* 1986). Less or insufficient soil moisture will reach the deeper subsurface layers of the soil where the roots of woody plants dominate, thus putting the trees at a disadvantage (Ward, Wiegand & Getzin 2013). The disturbance created by the selective removal of grass cover by heavy grazing allows the infiltration and percolation of water to deeper soil layers exploited by the roots of woody vegetation, conferring a competitive advantage to trees (Knoop & Walker 1985; Kambatuku, Cramer & Ward 2011). As a result, woody vegetation may then recruit *en masse* in the patches opened up by grazing disturbances (Ward 2005; Kambatuku 2010).

## 5.6. Conclusion

We showed that seed ingestion by goats improves seedling emergence. In addition, the fire treatment (used to remove the above-ground grasses) and mowed-grass treatment (used to simulate the indirect effect of grazing) played an important role by reducing grass competition with tree seedlings for resources. Regardless of the relatively low seedling emergence, seedling survival and recruitment, this study shows that the direct or indirect effects of gut passage, grass competition and precipitation were important in the recruitment of *D. cinerea* and *A. nilotica* seedlings, and may consequently contribute to woody plant encroachment. Overall, we showed in this experiment that the passage of seeds through the guts of different animals is not really a significant issue for woody plant encroachment. However, it was clear that interactions of various other factors (such as fire) and their direct and indirect effects may lead to woody plant encroachment.

## **5.7. Acknowledgements**

The study was financed by National Research Foundation (NRF) and International Foundation for Science (IFS). We thank the following students: Marvin Mavhunga, Malose Matlou and Veronica Rakoeana and Rangelands Management personnel for their assistance during the experiment. The assistance with statistical analysis of the Agricultural Research Council (ARC) Biometry Division, particularly Frikkie Calitz and Eric Mathebula, is greatly appreciated.

## 5.8. References

- Bodmer, R.E. & Ward, D. (2006) Frugivory in large mammalian herbivores. *Large Herbivore Ecology, Ecosystem Dynamics and Conservation*. (eds K. Danell, P. Duncan, R. Bergstrom & J. Pastor), pp. 232-260. Cambridge University Press, Cambridge, UK.
- Brown, N.A.C. & de Booyesen, P.V. (1967) Seed germination and seedling growth of two *Acacia* species under field conditions in grassveld. *South African Journal of Agricultural Science*, **10**, 659-666.
- Brown, J.R. & Archer, S. (1987) Woody plant seed dispersal and gap formation in a North American subtropical savanna woodland: the role of domestic herbivores. *Plant Ecology*, **73**, 73-80.
- Brown, J., Scholtz, C.H., Janeau, J-L., Grellier, S. & Podwojewski, P. (2010) Dung beetles (Coleoptera: Scarabaeidae) can improve soil hydrological properties. *Applied Soil Ecology*, **46**, 9-16.
- Bush, J.K. & Van Auken, O.W. (1995) Woody plant growth related to planting time and clipping of a C<sub>4</sub> grass. *Ecology*, **76**, 1603-1609.
- Campbell, M.L. & Clarke, P.J. (2006) Seed dynamics of resprouting shrubs in grassy woodlands: seed rain, predators and seed loss constrain recruitment potential. *Austral Ecology*, **31**, 1016-1026.
- Coe, M. & Coe, C. (1987) Large herbivores, *Acacia* trees and bruchid beetles. *South African Journal of Science*, **83**, 624-635.

- Coffin, D.P. & Lauenroth, W.K. (1990) A gap dynamics simulation model of succession in a semi-arid grassland. *Ecological Modelling*, **49**, 229-266.
- Coughenour, M.B. & Detling, J.K. (1986) *Acacia tortilis* seed germination responses to water potential and nutrients. *African Journal of Ecology*, **24**, 203-205.
- Cramer, M.D. & Chimphango, S.B.M. & Cauter, A.V. & Waldram, M.S. & Bond, W.J. (2007) Grass competition induces N<sub>2</sub> fixation in some species of African *Acacia*. *Journal of Ecology*, **95**, 1123-1133.
- Danell, K., Bergstrom, R., Edenius, L. & Ericsson, G. (2003) Ungulates as drivers of tree population dynamics at module and genet levels. *Forest Ecology and Management*, **181**, 67-76.
- Dinerstein, E. & Wemmer, C.M. (1988) Fruits rhinoceros eat: dispersal of *Trewia nudiflora* in lowland Nepal. *Ecology*, **69**, 1768-1774.
- Frost, P., Medina, E., Menaut, J.C., Solbrig, O., Swift, M. & Walker, B. (1986) Responses of savannas to stress and disturbances. *Biology International*, **10**, 1-8.
- Gardener, C.J., Mclvor, J.G. & Jansen, A. (1993) Passage of legume and grass seeds through the digestive tract of cattle and their survival in feces. *Journal of Applied Ecology*, **30**, 63-74.
- Goheen, J.R., Palmer, T.M., Keesing, F., Riginos, C. & Young, TP. (2010) Large herbivores facilitate savanna tree establishment via diverse and indirect pathways. *Journal of Animal Ecology*, **79**, 372-382.

- Grellier, S., Barot, S., Janeau, J. & Ward, D. (2012) Grass competition is more important than seed ingestion by livestock for *Acacia* recruitment in South Africa. *Plant Ecology*, **213**, 899-908.
- Hagos, M.G. & Smit, G.N. (2005) Soil enrichment by *Acacia mellifera* subsp. *detinens* on nutrient-poor sandy soil in a semi-arid southern African savanna. *Journal of Arid Environments*, **61**, 47-59.
- Henery, M.L. & Westoby, M.. (2001) Seed mass and seed nutrient content as predictors of seed output variations between species. *Oikos*, **92**, 479-490.
- Herrera, C.M., Jordano, P., López-Soria, L. & Amat, J. (1994) Recruitment of a mast-fruiting, bird dispersed tree: bridging frugivore activity and seedling establishment. *Ecological Monographs*, **64**, 315-344.
- Higgins, S.I., Bond, W.J. & Trollope, W.S.W. (2000) Fire, resprouting and variability: a recipe for grass-tree coexistence in savanna. *Journal of Ecology*, **88**, 213-229.
- Hoffman, T. & Ashwell, A. (2001) *Nature Divided: Land Degradation in South Africa*. University of Cape Town Press, Cape Town, South Africa.
- Jakobsson, A. & Eriksson, O. (2000) A comparative study of seed number, seed size, seedling size and recruitment in grassland plants. *Oikos*, **88**, 494-502.
- James, J.J., Svejcar., T.J. & Rinella, M.J. (2011) Demographic processes limiting seedling recruitment in arid grassland restoration. *Journal of Applied Ecology*, **48**, 961-969.

- Janzen, D.H. (1984) Dispersal of small seeds by big herbivores: foliage is the fruit. *American Naturalist*, **123**, 338-353.
- Jeltsch, F., Milton, S.J., Dean, W.R.J. & van Rooyen, N. (1996) Tree spacing and coexistence in semiarid savannas. *Journal of Ecology*, **84**, 583-595.
- Kambatuku, J.R. (2010) *Ecophysiology of Encroaching Acacia mellifera in Intra-and Inter-specific Interactions*. PhD thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa.
- Kambatuku, J.R., Cramer, M.D. & Ward, D. (2011) Savanna tree-grass competition is modified by substrate type and herbivory. *Journal of Vegetation Science*, **22**, 225-237.
- Kambatuku, J.R., Cramer, M.D. & Ward, D. (2011) Intraspecific competition between shrubs in a semi-arid savanna. *Plant Ecology*, **212**, 701-713.
- Kanz, W.A. (2001) *Seed and Seedling Dynamics of Certain Acacia Species as Affected by Herbivory, Grass Competition, Fire and Grazing Systems*. MSc thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa.
- Kgosikoma, O.E., Harvie, B.A. & Mojeremane, W. (2012) Bush encroachment in relation to rangeland management systems and environmental conditions in Kalahari ecosystem of Botswana. *African Journal of Agricultural Research*, **15**, 2312-2319.
- Knoop, W. & Walker, B.H. (1985) Interactions of woody and herbaceous vegetation in a southern African savanna. *Journal of Ecology*, **73**, 235-253.



- Kraaij, T. & Ward, D. (2006) Effects of rain, nitrogen, fire and grazing on tree recruitment and early survival in bush-encroached savanna. *Plant Ecology*, **186**, 235-246.
- Lamprey, H.F. (1983) Pastoralism yesterday and today: the overgrazing problem. *Tropical Savanna: Ecosystems of the World* (ed F. Bouliere), pp. 643-666. Elsevier, Amsterdam, The Netherlands.
- Leishman, M.R., Wright, I.J., Moles, A.T. & Westoby, M. (2000) The evolutionary ecology of seed size. *Seeds – The Ecology of Regeneration in Plant Communities* (ed M. Fenner), pp. 31-57. CAB International, Wallingford, UK.
- Little, T.M. & Hills, F.J. (1972) *Statistical Methods in Agricultural Research*. pp. 93-101. University of California, Davis, CA, USA.
- Maclean, J.E., Goheen, J.R., Doak, D.F., Palmer, T.M. & Young, T.P (2011) Cryptic herbivores mediate the strength and form of ungulate impacts on a long-lived savanna tree. *Ecology*, **92**, 1626-1636.
- McNaughton, S.J. (1983) Compensatory plant growth as a response to herbivory. *Oikos*, **40**, 329-336.
- Meyer, S.E. & Pendleton, B.K. (2005) Factors affecting seed germination and seedling establishment of a long-lived desert shrub (*Coleogyne ramosissima*: Rosaceae). *Plant Ecology*, **178**, 171-187.
- Miller, M.F. & Coe, M. (1993) Is it advantageous for *Acacia* seeds to be eaten by ungulates? *Oikos*, **66**, 364-368.

- Miller, M.F. (1995) *Acacia* seed survival, seed germination and seedling growth following pod consumption by large herbivores and seed chewing rodents. *African Journal of Ecology*, **33**, 194-210.
- Moles, A.T. & Westoby, M. (2002) Seed addition experiments are more likely to increase recruitment in larger-seeded species. *Oikos*, **99**, 241-248.
- Moles, A.T., Ackerly, D.D., Webb, C.O., Tweddle, J.C., Dickie, J.B. & Westoby, M. (2005) A brief history of seed size. *Science*, **307**, 576-580.
- Mucina, L. & Rutherford, M.C. (2006) *The Vegetation of South Africa, Lesotho and Swaziland*. Strelitzia 19, South African National Biodiversity Institute, Pretoria, South Africa.
- Nathan, R. & Muller-Landau, H.C. (2000) Spatial pattern of seed dispersal, their determinants and consequences of recruitment. *Trends in Ecology and Evolution*, **15**, 278-285.
- O'Connor, T.G. (1995) *Acacia karroo* invasion of grassland: environmental and biotic effects influencing seedling emergence and establishment. *Oecologia*, **103**, 214-223.
- Or, K. & Ward, D. (2003) Three-way interactions between *Acacia*, large mammalian herbivores and bruchid beetles - a review. *African Journal of Ecology*, **41**, 257-265.
- Or, K. & Ward, D. (2007) Maternal effects on the life histories of bruchid beetles infesting *Acacia raddiana* in the Negev desert, Israel. *Entomologia Experimentalis et Applicata*, **122**, 165-170.

- Rodriguez-Peréz J., Wiegand, K. & Ward, D. (2011) Interaction between ungulates and bruchid beetles and its effect on *Acacia* trees: modeling the costs and benefits analysis of seed dispersal to plant demography. *Oecologia*, **167**, 97-105.
- Rohner, C.J. & Ward, D. (1999) Large mammalian herbivores and the conservation of arid *Acacia* stands in the Middle East. *Conservation Biology*, **13**, 1162-1171.
- Sankaran, M., Ratnam, J. & Hanan, N.P. (2004) Tree-grass co-existence in savannas revisited – insights from an examination of assumptions and mechanisms invoked in existing models. *Ecology Letters*, **7**, 480-490.
- SAS Institute. (2002) *SAS/STAT User's Guide*, Version 9.2. SAS Institute, Cary, NC, USA.
- Scholes, R.J. & Archer, S.R. (1997) Tree-grass interactions in savannas. *Annual Review of Ecology and Systematics*, **28**, 517–544.
- Schupp, E.W. & Fuentes, M. (1995) Spatial patterns of seed dispersal and the unification of plant population ecology. *Ecoscience*, **2**, 267-275.
- Schupp, E.W., Gomes, J.M., Jimenez, J.E. & Fuentes, M. (1997) Dispersal of *Juniperus occidentalis* (Western Juniper) seeds by frugivorous mammals on Juniper Mountain, southeastern Oregon. *Great Basin Naturalist*, **57**, 74-78.
- Schwinning, S., Davis, K., Richardson, L. & Ehleringer, J.R. (2002) Deuterium-enriched irrigation indicates different forms of rain use in shrub/grass species of the Colorado Plateau. *Oecologia*, **130**, 345-355.

- Seabloom, E.W., Borer, E.T., Boucher, V.L., Burton, R.S., Cottingham, K.L., Goldwasser, L., Gram, W.K., Kendall, B.E. & Micheli, F. (2003) Competition, seed limitation, disturbances, and reestablishment of California native annual forbs. *Ecological Applications*, **13**, 575-592.
- Shapiro, S.S. & Wilks, M.B. (1965) An analysis of variance test for normality (complete samples). *Biometrika*, **52**, 591-611.
- Sharam, G., Sinclair, A.R.E. & Turkington, R. (2006) Establishment of broad-leaved thickets in Serengeti, Tanzania: the influence of fire, browsers, grass competition and elephants. *Biotropica*, **38**, 599-605.
- Skarpe, C. (1991) Spatial patterns and dynamics of woody vegetation in an arid savanna. *Journal of Vegetation Science*, **2**, 565-572.
- Smith, C.C. & Fretwell, S.D. (1974) The optimal balance between size and number of offspring. *American Naturalist*, **108**, 499-506.
- Snedecor, G.W. & Cochran, W.G. (1967) *Statistical Methods*, 6th ed. Iowa State University Press, Ames, IA, USA.
- Tews, J., Moloney, K. & Jeltsch, F. (2004) Modeling seed dispersal in a variable environment: a case study of the fleshy-fruited savanna shrub *Grewia flava*. *Ecological Modelling*, **175**, 65-76.

- Tjelele, T.J., Dziba, L.E. & Pule, H.T. (2012) Recovery and germination of *Dichrostachys cinerea* seeds fed to goats (*Capra hircus*). *Rangeland Ecology and Management*, **65**, 105-108.
- Traveset, A., Bermejo, T. & Willson, M. (2001) Effect of manure composition on seedling emergence and growth of two common shrub species of Southeast Alaska. *Plant Ecology*, **155**, 29-34.
- Turnbull, L.A., Rees, M. & Crawley, M.J. (1999) Seed mass and the competition/colonization trade-off: a sowing experiment. *Oikos*, **88**, 899-912.
- Valentin, C. & Bresson, L.M. (1992) Morphology, genesis and classification of soil crusts in loamy and sandy soils. *Geoderma*, **55**, 225-245.
- van Auken, O.W. (2009) Causes and consequences of woody plant encroachment into western North American grassland. *Journal of Environmental Management*, **90**, 2931-2942.
- Walter, H. (1939) Grassland, savanne und busch der arideren Teile Afrikas in ihrer ökologischen Bedingtheit. *Jahrbucher für Wissenschaftliche Botanik*, **87**, 750-860.
- Ward, D. (2005) Do we understand the causes of bush encroachment in African savannas? *African Journal of Range and Forage Science*, **22**, 101-106.
- Ward, D. & Esler, K.J. (2010) What are the effects of substrate and grass removal on recruitment of *Acacia mellifera* seedlings in a semi-arid environment? *Plant Ecology*, **212**, 245-250.
- Ward, D., Wiegand, K. & Getzin, S. (2013) Walter's two-layer hypothesis revisited: back to the roots! *Oecologia*, **172**, 617-630.

- Weitbrecht, K., Müller, K. & Leubner-Metzger, G. (2011) First off the mark: early seed germination. *Journal of Experimental Botany*, **62**, 3289-3309.
- Westoby, M., Leishman, M. & Lord, J. (1996) Comparative ecology of seed size and dispersal. *Philosophical Transactions of the Royal Society B*, **351**, 1309-1318.
- Whitacre, M.K. & Call, C.A. (2006) Recovery and germinability of native seed fed to cattle. *Western North American Naturalist*, **66**, 121-128.
- Wiegand, K., Ward, D. & Saltz, D. (2005) Multi-scale patterns in an arid savanna with a single soil layer. *Journal of Vegetation Science*, **16**, 311-320.
- Wiegand, K., Saltz, D. & Ward, D. (2006) A patch-dynamics approach to savanna dynamics and woody plant encroachment – Insights from an arid savanna. *Perspectives in Plant Ecology, Evolution and Systematics*, **7**, 229-242.
- Wigley, B.J., Bond, W.J. & Hoffman, M.T. (2009) Bush encroachment under three contrasting land-use practices in a mesic South African savanna. *African Journal of Ecology*, **47**, 62-70.
- Wigley, B.J., Bond, W.J. & Hoffman, M.T. (2010) Thicket expansion in a South African savanna under divergent land use: local vs. global drivers? *Global Change Biology*, **16**, 964-976.
- Wilson, J.B. (1988) Shoot competition and root competition. *Journal of Applied Ecology*, **25**, 279-296.

Wilson, T.B. & Witkowski, E.T.F. (1998). Water requirements for germination and early seedling establishment in four African savanna woody plant species. *Journal of Arid Environments*, **38**, 541-550.

**Table 1:** The seedling emergence trial consisted of a completely randomized design with three replicates per treatment: passage through goats or cattle or unpassed/untreated seeds, dung, grass competition, fire and trampling.

<b>Gut passage through goats</b>		<b>Gut passage through cattle</b>		<b>Unpassed/untreated seeds (i.e. not ingested by goats or cattle)</b>	
Dung	Control (no dung)	Dung	Control (no dung)	Dung	Control (no dung)
Fire	Control (no fire)	Fire	Control (no fire)	Fire	Control (no fire)
Grass competition	Mowed grass	Grass competition	Mowed grass	Grass competition	Mowed grass
Trampling	Control (no trampling)	Trampling	Control (no trampling)	Trampling	Control (no trampling)



**Table 2:** *F* values and *P* values for the effects of the seven treatments and their interactions and their interactions on seedling emergence, seedling survival and seedling recruitment. Dashes (-) = nonsignificant.

Treatments	Dry season						Wet season					
	Germination		Survival		Recruitment		Germination		Survival		Recruitment	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Animal spp.	61.2	<.0001	51.3	<.0001	60.9	<.0001	14.1	<.0001	8.8	0.0003	11.8	<.0001
Seed spp.	11.0	0.0012	10.4	0.0016	10.3	0.0017	-	-	-	-	-	-
Fire	41.1	<.0001	40.4	<.0001	48.9	<.0001	29.0	<.0001	28.7	<.0001	25.1	<.0001
Animal spp. × grass × fire	4.2	0.0167	3.6	0.0317	5.5	0.0052	-	-	-	-	-	-
Animal spp. × dung × grass	4.5	0.0136	3.9	0.0227	3.6	0.0312	-	-	-	-	-	-
Animal spp. × seed spp. × dung × fire	3.8	0.0236	-	-	-	-	-	-	-	-	-	-
Animal spp. × seed spp. × grass × fire	3.7	0.0279	-	-	-	-	-	-	-	-	-	-
Seed spp. × dung × grass	-	-	-	-	-	-	6.9	0.0099	4.8	0.0305	8.8	0.0036
Seed spp. × dung × fire	-	-	7.0	0.0092	-	-	-	-	-	-	-	-

## 5.9. Figure Legends

Fig. 1. The interaction effects of animal species (goats, cattle), unpassed/untreated seeds (i.e. not ingested), grass (G) (and mowed grass (Gc)), fire (F) (and no fire (Fc)) on seedling recruitment during the dry season. Bars represent standard errors (S.E). Same letters on the bars mean that  $P > 0.05$ . Fisher's *post hoc* test was used.

Fig. 2. The interaction effects of animal species (goats, cattle), unpassed/untreated seeds (i.e. not ingested), dung (D) (and no dung (Dc)), and grass (G) (and mowed grass (Gc)) on seedling recruitment during the dry season. Bars represent standard errors (S.E). Same letters on the bars mean that  $P > 0.05$ . Fisher's *post hoc* test was used.

Fig. 3. The interaction effects of animal species (goats, cattle), unpassed/untreated seeds (i.e. not ingested), seed species (*A. nilotica*, *D. cinerea*), grass (G) (and mowed grass (Gc)), fire (F) (and no fire (Fc)) on seedling emergence during the dry season. Bars represent standard errors (S.E). Same letters on the bars mean that  $P > 0.05$ . Fisher's *post hoc* test was used.

Fig. 4. The interaction effects of seed species (*A. nilotica*, *D. cinerea*), dung (D) (and no dung (Dc)), fire (F) (and no fire (Fc)) on seedling survival during the dry season. Bars represent standard errors (S.E). Same letters on the bars mean that  $P > 0.05$ . Fisher's *post hoc* test was used.

Fig. 5. The interaction effects of seed species (*A. nilotica*, *D. cinerea*), dung (D) (and no dung (Dc)), grass (G) (and mowed grass (Gc)) on seedling emergence during the wet season. Bars represent standard errors (S.E). Same letters on the bars mean that  $P > 0.05$ . Fisher's *post hoc* test was used.

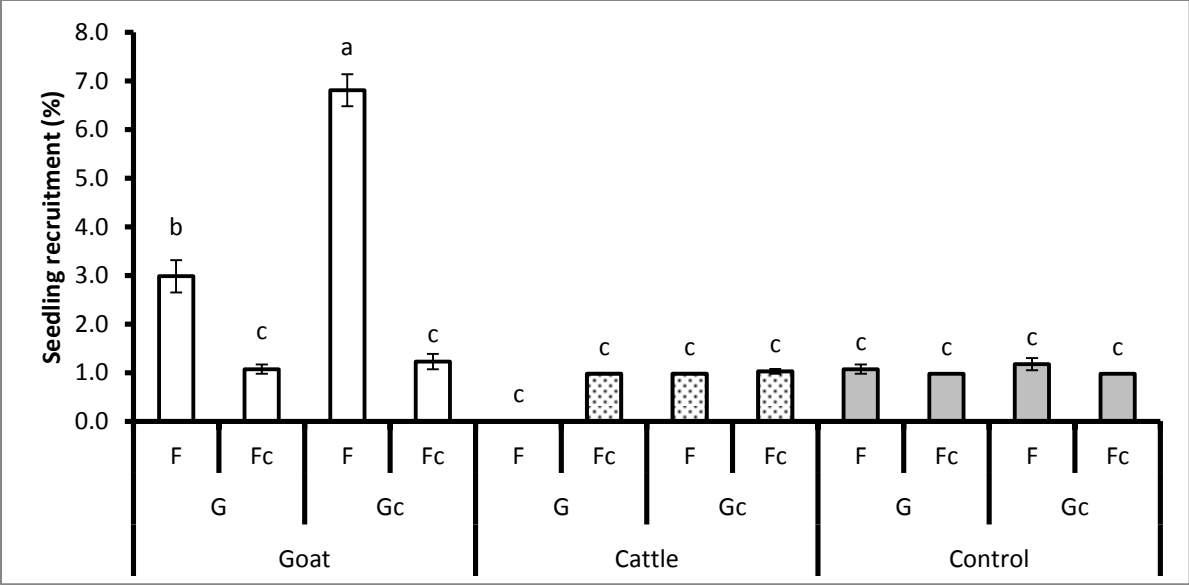


Fig. 1.

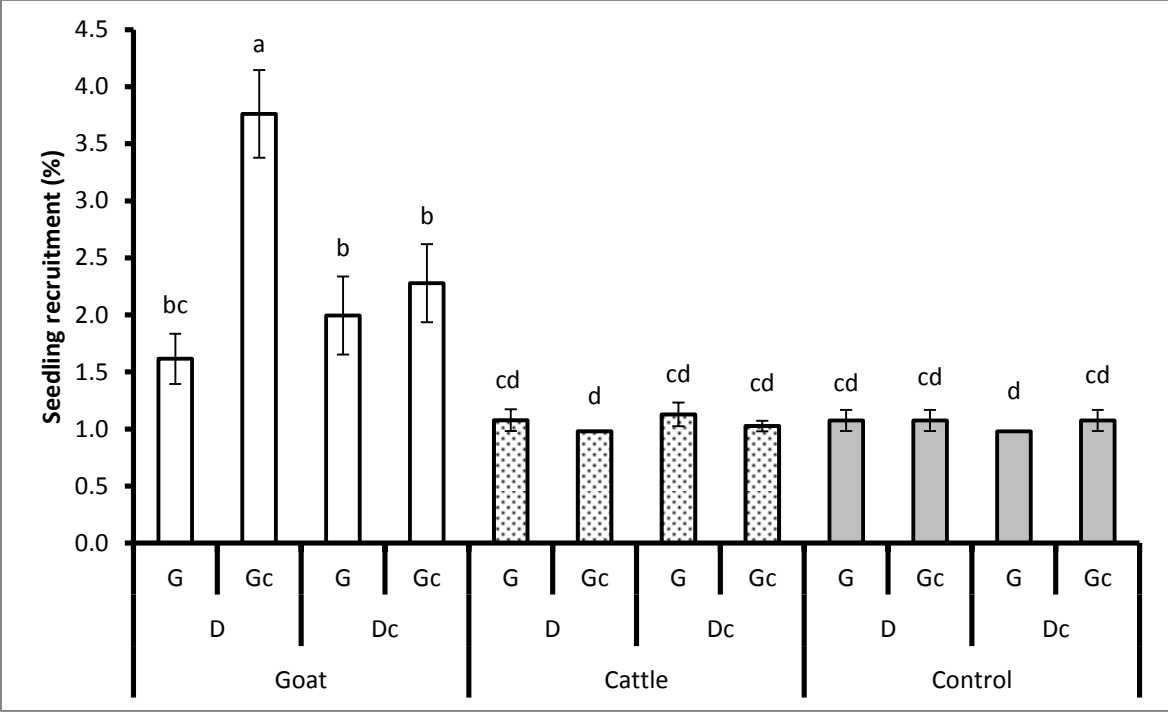


Fig. 2.

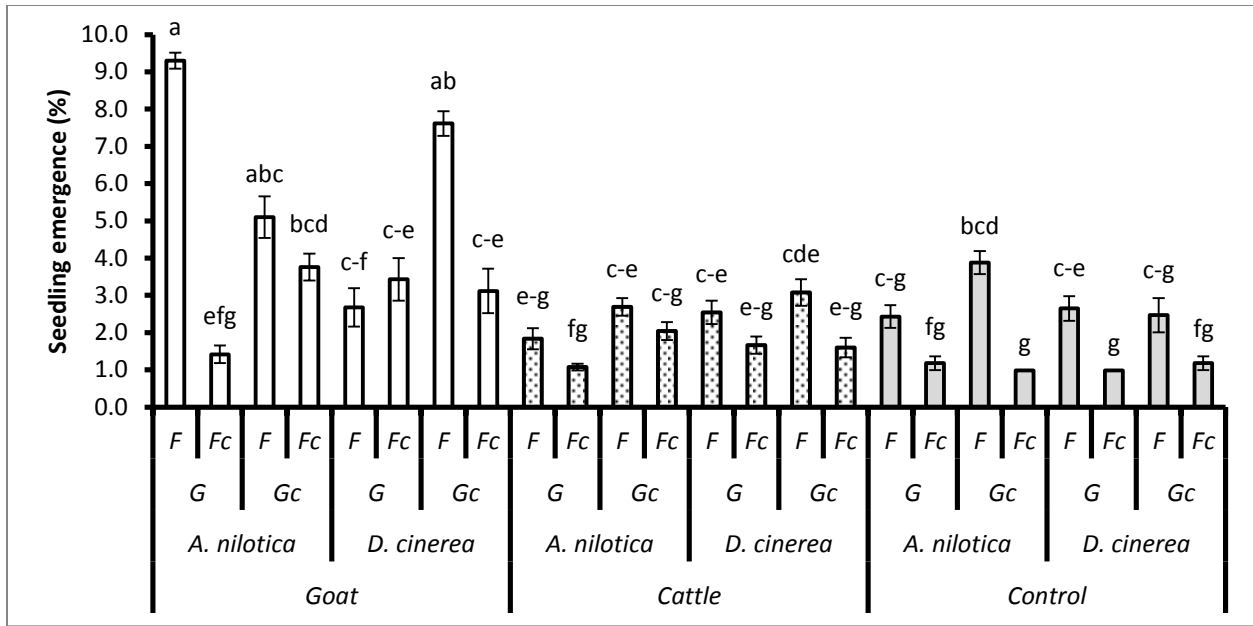


Fig. 3.

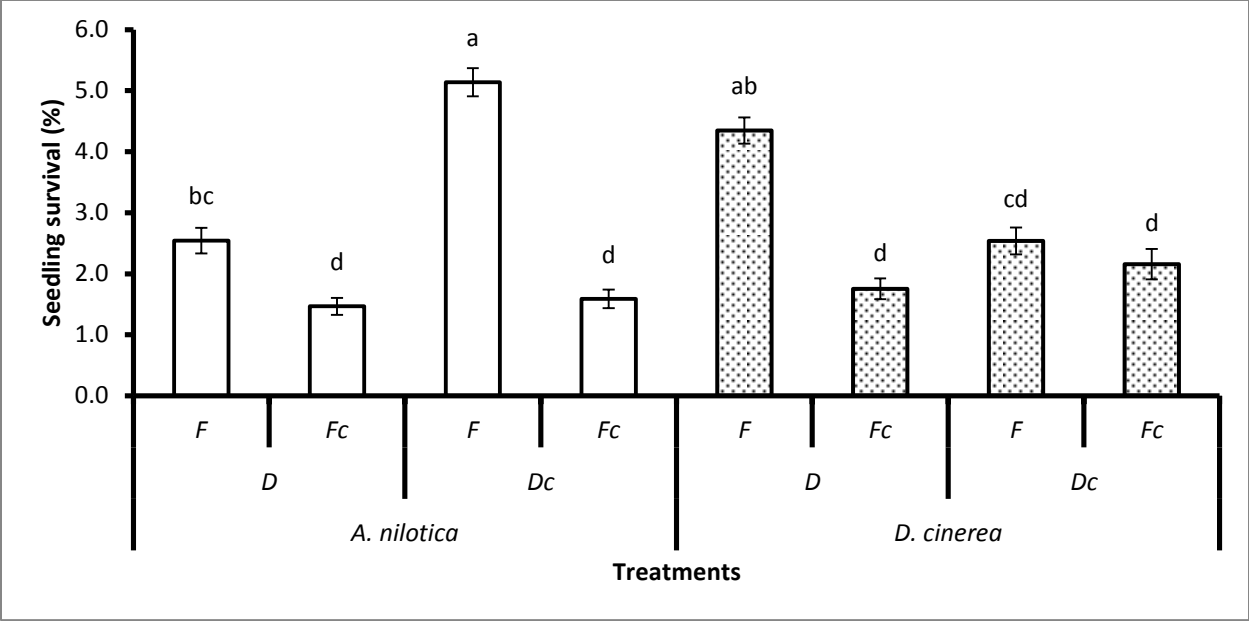


Fig. 4.

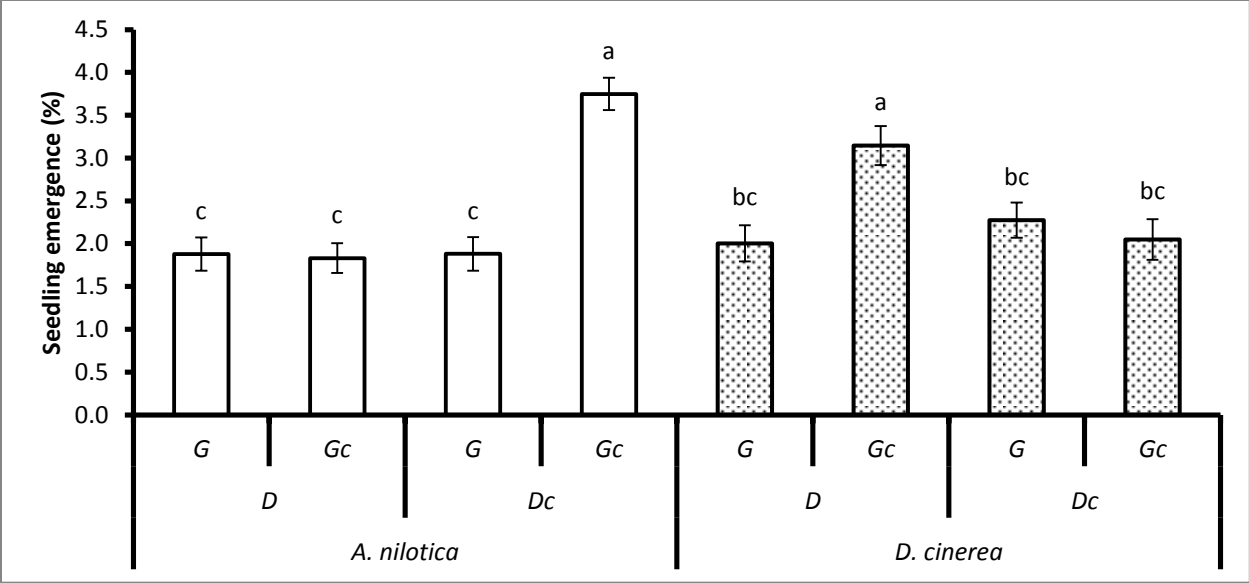


Fig. 5.



## Chapter 6

### 6.1. Synthesis

The main aim of this study was to understand several underlying factors in woody plant encroachment, specifically animal seed recovery, seedling emergence, establishment, survival and recruitment. In this chapter, I summarize and integrate (using a seed fate model) the research results reported in this study (chapter 3, 4 and 5) and consider the most fruitful routes for further research. I focused on seedling emergence and recruitment, which are the most important factors for determining the global effects of different factors and their interaction on woody plant encroachment from the seed to the seedling stage (Salazar 2010, Ward and Esler 2010, Grellier et al. 2012). I found in this study that the passage of seeds through the digestive systems of different animals improves seedling emergence. However, in general, passage of seeds through animal digestive systems is not really a significant issue for woody plant encroachment. Grass competition has been shown to be the most important factor for recruitment of *A. nilotica* and *D. cinerea* seedlings (see e.g. Ward 2005, Riginos 2009, Grellier *et al.* 2012 for examples from other *Acacia* species).

This study did not support the hypothesis that dung provides a suitable nutritive medium for germination and seedling establishment (Coughenour and Detling 1986, Dinerstein and Wemmer 1988). The results showed that seeds planted without dung (either buried 2 cm in the soil or on top of the soil) germinated significantly more than seeds planted with dung. Seeds that

were softened by gut passage became harder than untreated seeds when exposed to the sun, which created a hard layer (a similar result was obtained by Grellier et al. 2012). The seedling emergence trial began during the dry season (as is the case in *Acacia* and *Dichrostachys* species that fruit in April-September), with no water applied, causing the dung to become dry and hard (Coe and Coe 1987, Miller and Coe 1993). This suggests that a positive effect of livestock may be to disperse seeds away from the mother tree rather than dung fertilization *per se* (Grellier et al. 2012).

Understanding the interaction effects of animal species (large-bodied animals vs. small-bodied animals), associated diet quality (low-quality vs. high-quality) and seed characteristics (size, shape, hardness) (Jones and Simao Neto 1987, Shayo and Uden 1989, Gardener et al. 1993, Bodmer and Ward 2006, Whitacre and Call 2006) that influence seed recovery, viability and seedling emergence may aid attempts to understand the causes and management of woody plant encroachment. In Chapter 3, the effects of diet quality influenced both seed recovery and germination, which suggested that animals fed seeds mixed with high-quality diet (e.g. *Medicago sativa* hay) are most likely to pass through the digestive tract intact and remain viable. In both experiment one (goats vs. sheep) and experiment two (goats vs. cattle), the percentage germination of seeds that passed through the digestive tract of animals was higher than of unpassed/untreated seeds. Hard-coated seeds such as those of *D. cinerea* and *A. nilotica* are usually resistant to damage during chewing and rumination (Gardener et al. 1993, Rohner and Ward 1999, Whitacre and Call 2006), which may have played a role in high seed recovery and seed germination. Thus, all three factors (animal species, associated diet quality and seed characteristics) both alone or in combination are important in recovery of viable *D. cinerea* and *A. nilotica* seeds.

## SEED FATE MODEL

Seed species	<i>A. nilotica</i>		<i>D. cinerea</i>		<i>A. nilotica</i>		<i>D. cinerea</i>				Notes
Animal spp.	Goat	Sheep	Goat	Sheep	Goat	Cattle	Goat	Cattle			
Seed rain (n)	1000	1000	1000	1000	1000	1000	1000	1000			<b>Chapter 3</b> , cattle consumed all <i>A. nilotica</i>
Seeds consumed (n)	628	940	1000	1000	820	1000	961	1000			& <i>D. cinerea</i> seeds.
Seeds retrieved											<b>Chapter 3</b> , more seeds retrieved from
after gut passage (%)	41.5	38.7	31.2	40.5	31.3	47.7	32.0	50.3			cattle compared to goats.
Seeds retrieved											<b>Chapter 4</b> , more seeds were retrieved from
after gut passage (%)	-	-	-	-	11.7	37.8	13.2	22.9			cattle compared to goats.

Diet	<i>D. eriantha</i>		<i>M. sativa</i>		<i>D. eriantha</i>		<i>M. sativa</i>		Goat	Cattle	Notes
Seed spp.	<i>A. nilotica</i>	<i>D. cinerea</i>	<i>A. nilotica</i>	<i>D. cinerea</i>	<i>A. nilotica</i>	<i>D. cinerea</i>	<i>A. nilotica</i>	<i>D. cinerea</i>			<b>Chapter 3</b> , diet quality and animal species
Germinated (%)	9.4	6.8	2.5	6.7	5.4	10.6	4.1	6.1	14.1	9.3	had a significant effect on germination.
Animal spp.	Goat		Cattle		Control (untreated seeds)						<b>Chapter 4</b> , data for seeds retrieved from
Planting method	F3	L4	F3	L4	F3	L4					goats and cattle in the first three days (F3)
Emergence (%) ND	10.2	24.9	4.9	16.0	4.36	5.21					and last four days (L4) and planted with
Recruitment (%)	9.2	24.7	5.1	16.2	4.2	4.9					no dung (ND) – seedling emergence.
											<b>Chapter 4</b> , no dung seedling recruitment.

Animal spp.	Goat				Cattle				Control (untreated seeds)				Notes
Grass/mowed	Grass (G)		Mowed grass (Gc)		Grass (G)		Mowed grass (Gc)		Grass (G)		Mowed grass (Gc)		<b>Chapter 5</b> , shows the effect of grass
Fire/no fire	Fire	No fire	Fire	No fire	Fire	No fire	Fire	No fire	Fire	No fire	Fire	No fire	competition on seedling emergence &
Emergence (%)	4.0	1.1	8.7	1.3	0.0	1.0	1.0	1.2	1.1	1.0	1.2	1.0	recruitment, especially for seeds
Recruitment (%)	3.0	1.1	6.9	1.2	0.0	1.0	1.0	1.0	1.1	1.0	1.2	1.0	retrieved from goats.
Recruitment (%) ND	2.0	-	2.3	-	1.1	-	1.0	-	1.0	-	1.1	-	<b>Chapter 5</b> , non-significant seedling
													recruitment with addition of dung, and
													effect of grass competition

Cattle should be more effective dispersers than goats because they are larger and thus the seeds spend more time in their guts, causing more scarification of the hard-coated seeds of *A. nilotica* and *D. cinerea* to occur (Bodmer and Ward 2006). Additionally, cattle presumably walk further than goats do, and may disperse the seeds further away. Our pen study on the recovery and germination of *D. cinerea* seeds fed to goats (Chapter 2) showed that passage of *D. cinerea* through the digestive tract of goats remained viable and had substantial germination potential. Although the viability of seeds that passed through the gut of goats was lower than mechanically scarified seeds, it was nearly double that of untreated seeds (i.e. with no seed passage through the digestive tract of goats). While this study showed great potential for goats to facilitate woody plant encroachment through dispersal of viable seeds, the mechanisms involved with woody plant encroachment need to be better understood.

In seeking to understand mechanisms such as animal species, associated diet quality and seed characteristics on seed recovery and seedling emergence, I conducted an experiment on the effects of associated diet quality on germination of *D. cinerea* and *A. nilotica* fed to ruminants (Chapter 3). The results in chapter 3 showed that large-bodied animals conferred higher seed recovery than smaller animals (in this case, seed recovery from cattle of *D. cinerea* and *A. nilotica* was greater than from goats), which was consistent with the results of Miller (1995) and Bodmer and Ward (2006). In addition, relatively high numbers of seeds were retrieved from goats (32%), which show the important role that goats can play in the retrieval of viable *D. cinerea* and *A. nilotica* seeds (see Chapter 3).

Retrieved intact and viable seeds by herbivores do not guarantee germination, seedling survival and seedling recruitment under natural conditions (Nathan and Muller-Landau 2000).

Seed germination, seedling establishment and recruitment are prerequisites for increasing tree abundance and are influential in the process of woody plant encroachment (Kraaij and Ward 2006, Salazar 2010). I therefore conducted two field experiments on the effects of gut passage and dung fertilization on seedling recruitment of *D. cinerea* and *A. nilotica* seeds (Chapter 4) and on the effects of seed ingestion by livestock, trampling and dung fertilization, grass competition and fire on seedling recruitment of *D. cinerea* and *A. nilotica* of woody plant species (Chapter 5). The results in chapter 4 showed that passage through the digestive tracts of goats and cattle, especially seeds retrieved during the last four days, played a significant role in the emergence of *D. cinerea* and *A. nilotica* (see also Rohner and Ward 1999, Grellier et al. 2012). Bodmer and Ward (2006) reported a positive correlation between germination and size of herbivores that ingest the seeds. Miller (1995) also reported that seed dispersal and germination usually, but not always, increased in larger herbivores. Interestingly, in my study, a significantly higher emergence percentage was found in goats (21.8%) than cattle (8.0%). The result from my study probably occurred because longer retention time of seeds in the digestive tract of large-bodied animals such as cattle may result in damaged seeds, possibly compromising germination. It is not only the allometric scaling of scarification time to herbivore mass (Robbins 1993) that may result in higher seed recovery and germination but the interactions of other factors such as seed characteristics (hardness, size and shape) and associated diet that play a vital role (see Chapter 3).

A better understanding of the interaction effects of gut passage by livestock, dung fertilization, fire and trampling on seedling recruitment of *D. cinerea* and *A. nilotica* will aid in developing management strategies of woody plant encroachment. The results from chapter 5 showed that a significantly higher seedling emergence percentage was obtained for goats

(3.94%) than cattle (1.97%), which was consistent with the results of chapter 4, and with the results of Miller (1995). Most importantly, seedling recruitment of *A. nilotica* and *D. cinerea* increased with fire and grass mowing treatments. Grasses are known to be superior competitors for resources (light, water and nutrients) than tree seedlings (Knoop and Walker 1985, Ward and Esler 2010, Ward et al. 2013). Significantly higher germination, seedling survival and recruitment of *A. nilotica* and *D. cinerea* were most probably due to reduced or no grass cover (O'Connor 1995, Campbell and Clarke 2006), regardless of whether it was caused by mowing or fire (see also Grellier et al. 2012). This study suggested that interactions of seed recovery by animals and seedling establishment in concert with environmental factors (precipitation and temperature) and disturbance factors (fire and grass competition) appear to play an important role in increasing and maintaining woody plant cover.

## **6.2. Management implications**

Goats have been used for managing woody plant encroachment (O'Connor 1996, El Aich and Waterhouse 1999, Scogings and Mopipi 2008), yet their role in the dispersal of viable seeds was unknown (especially during the winter periods when the seeds form an important part of the diet of livestock due to their high nutritive value) (Coe and Coe 1987, Or and Ward 2004, van Auken 2009). It is evident from this study that domestic ruminants (goats, sheep and cattle) and the interactions of associated diet and seed species with different characteristics play an important role in the recovery of viable seeds; together these may facilitate woody plant encroachment by enhancing seedling emergence. *D. cinerea* and *A. nilotica* seeds remained in the gut of goats,

sheep and cattle for about 9 days (see Chapter 2, 3 and 4). For this reason, herbivores have the potential to disperse viable seeds away from the parent tree, which will favour spreading of seeds where competition with adult trees is minimal (Miriti 2006, Kambatuku et al. 2011, Grellier et al. 2012). I recommend that animals consuming woody plant seeds should be restricted to paddocks to reduce the possibility of woody plant encroachment throughout the ranch. It must be noted that animals will consume pods of different woody plants; hence, they can only be restricted to paddocks for about 9 days (see Chapters 2 and 3).

The use of fire and mowed-grasses and adequate rainfall may result in an increased seed germination and seedling recruitment. These results will significantly increase our understanding of mechanisms (especially on seed recovery by ruminants) underlying woody plant encroachment and aid in developing management strategies.

### **6.3. Future research**

The increasing rate and extent of woody plant encroachment in grasslands and savannas remains a challenge to farmers interested in grass production (Ward 2005, Wigley et al. 2010). Future studies must focus on developing management strategies of woody plant encroachment. The dynamics underlying woody plant encroachment, specifically on seed recovery by domestic ruminants, alone or in combination with other factors (associated diet, seed characteristics, dung nutrients, fire, grass competition and season) in this study yielded promising results. Robbins (1993), Bodmer and Ward (2006) and Castro et al. (2008) reported that allometric scaling of digestion time to herbivore mass may result in increased germination. Interestingly, the results in

Chapter 4 and 5 showed the opposite (ingestion by goats resulted in higher seedling emergence than by cattle). We therefore need to clearly determine the effect of body size and/or age within the same animal species, especially of goats (as the main browsers of seed pods) on seed recovery and seedling emergence. Such knowledge will clarify whether different animal body sizes and ages also contribute to the recovery of viable woody plant seeds.

### **Can browsing-fire interactions contribute towards management of woody plant encroachment?**

Prescribed burning is an important ecological factor for management of woody plant encroachment (Trollope 1980, Morton et al. 2010, Becerra et al. 2013). Goats have been used in controlling woody plant encroachment even in woody plant species heavily defended by secondary metabolites (Trollope 1980, El Aich and Waterhouse 1999), especially following mechanical clearing (O'Connor 1996, Scogings and Mopipi 2008). The application of strategic supplementation (supplemental nutrients) can enhance the capacity for detoxification and create opportunities to use goats as a tool to manage woody plant encroachment (Papachristou et al. 2005). Furthermore, provision of supplemental nutrients encourages browsers to consume plants that are usually avoided as a result of low quality and/or chemically defended plants (Titus et al. 2000, Provenza et al. 2003, Papachristou et al. 2005). Fire can be used to create burned patches during certain periods of the year, which can be used strategically to attract goats to underutilized plant species (Brockett et al. 2001, Vermeire et al. 2004). Plants that are chemically-defended tend to be less palatable and avoided (see e.g. Shrader et al. 2012). However, when these chemically defended plants have nutritious neighbours, they may be consumed (Palmer et al.



2003). Nutritious post-burn regrowth can serve as supplements that encourage browsing of chemically-defended plants (Brockett et al. 2001, Vermeire et al. 2004). The influence of chemical defence and nutrients separately will not provide adequate information on diet selection and foraging behavior of goats (Provenza et al. 2003). Therefore, understanding the interaction of secondary compounds and supplemental nutrients will be essential for developing management strategies of woody plant encroachment. Strategic supplement with nutrients such as proteins and energy (Dziba et al. 2007) may enable ruminants to consume more plant secondary metabolites in their forage, which may allow them to inflict more damage on woody plants.

The presence of plant secondary metabolites influences diet selection and foraging behaviour of animals (Rohner and Ward 1997, Dziba et al. 2006). Diet selection is further influenced by several factors including, among other factors, stocking density, grazing periods, seasonal variation in plant chemistry and availability of nutritious alternatives (Ward and Young 2002, Dziba et al. 2003, Provenza et al. 2003, Papachristou et al. 2005, Shrader et al. 2012). It is imperative to also focus the effects of stocking density on diet selection in an attempt to manage woody plant encroachment. Animal performance and production are further compromised by limited choice because of lower nutritional plant quality during the dry season as a result of seasonal effects of selective browsing (Foley and Moore 2005). This study showed that herbivores, especially goats, play a vital role in the dispersal of viable woody plant seeds, thereby contributing to woody plant encroachment. It was clear that there is no single cause of woody plant encroachment, but a combination of interacting factors. It is unlikely that most ranchers will be able to afford the expensive chemical treatments commonly used to combat

woody plant encroachment. Hence, we need to further explore the role of inexpensive combinations of fire and browsing to limit the spread of woody plants.

#### 6.4. References

- Becerra TA, Engle DM, Elmore RD, Fuhlendorf SD. 2013. Contrasting preference for grassland landscapes among population groups in the central and southern Great Plains. *Rangeland Ecology and Management* 66: 529-538.
- Bodmer RE, Ward D. 2006. Frugivory in large mammalian herbivores. In: Danell K, Duncan P, Bergstrom R, Pastor J (eds.). *Large Herbivore Ecology, Ecosystem Dynamics and Conservation*. Cambridge University Press, Cambridge, UK. pp 232-260.
- Brockett BH, Biggs HC, van Wilgen BW. 2001. A patch mosaic burning system for conservation areas in southern African savannas. *International Journal of Wildland Fire* 10: 169-183.
- Campbell ML, Clarke PJ. 2006. Seed dynamics of resprouting shrubs in grassy woodlands: seed rain, predators and seed loss constrain recruitment potential. *Austral Ecology* 31: 1016-1026.
- Castro SA, Bozinovic F, Jaksic FM. 2008. Ecological efficiency and legitimacy in seed dispersal of an endemic shrub (*Lithrea caustica*) by the European rabbit (*Oryctolagus cuniculus*) in central Chile. *Journal of Arid Environments* 72: 1164-1173.
- Coe M, Coe C. 1987. Large herbivores, *Acacia* trees and bruchid beetles. *South African Journal of Science* 83: 624-635.

- Coughenour MB, Detling JK. 1986. *Acacia tortilis* seed germination responses to water potential and nutrients. *African Journal of Ecology* 24: 203-205.
- Dinerstein E, Wemmer CM. 1988. Fruits rhinoceros eat: dispersal of *Trewia nudiflora* in lowland Nepal. *Ecology* 69: 1768-1774.
- Dziba LE, Scogings PF, Gordon IJ, Raats JG. 2003. Effects of season and breed on browse species intake rates and diet selection by goats in the False Thornveld of the Eastern Cape, South Africa. *Small Ruminant Research* 47: 17-30.
- Dziba LE, Hall JO, Provenza FD. 2006. Feeding behavior of lambs in relation to kinetics of 1,8-cineole dosed intravenously and into the rumen. *Journal of Chemical Ecology* 32: 391-408.
- Dziba LE, Provenza FD, Villalba JJ, Atwood SB. 2007. Supplemental energy and protein increase use of sagebrush by sheep. *Small Ruminant Research* 69: 203-207.
- El Aich A, Waterhouse A. 1999. Small ruminants in environmental conservation. *Small Ruminant Research* 34: 271-287.
- Foley W, Moore BD. 2005. Plant secondary metabolites and vertebrate herbivores – from physiological regulation to ecosystem function. *Current Opinion in Plant Biology* 8: 430-435.
- Gardener CJ, McIvor JG, Jansen A. 1993. Passage of legume and grass seeds through the digestive tract of cattle and their survival in feces. *Journal of Applied Ecology* 30: 63-74.

- Grellier S, Barot S, Janeau J, Ward D. 2012. Grass competition is more important than seed ingestion by livestock for *Acacia* recruitment in South Africa. *Plant Ecology* 213: 899-908.
- Jones RM, Simao Neto M. 1987. Recovery of pasture seed ingested by ruminants. 3. The effect of the amount of seed in the diet and of diet quality on seed recovery from sheep. *Australian Journal of Experimental Agriculture* 27: 253-256.
- Kambatuku JR, Cramer MD, Ward D. 2011. Intraspecific competition between shrubs in a semi-arid savanna. *Plant Ecology* 212: 701-713.
- Knoop W, Walker BH. 1985. Interactions of woody and herbaceous vegetation in a southern African savanna. *Journal of Ecology* 73: 235-253.
- Kraaij T, Ward D. 2006. Effects of rain, nitrogen, fire and grazing on tree recruitment and early survival in bush-encroached savanna. *Plant Ecology* 186: 235 –246.
- Miller MF, Coe M. 1993. Is it advantageous for *Acacia* seeds to be eaten by ungulates? *Oikos* 66: 364-368.
- Miller MF. 1995. *Acacia* seed survival, seed germination and seedling growth following pod consumption by large herbivores and seed chewing rodents. *African Journal of Ecology* 33: 194-210.
- Miriti M. 2006. Ontogenetic shift from facilitation to competition in a desert shrub. *Journal of Ecology* 94: 973-979.

- Morton LW, Regen E, Engle DM, Miller JR, Harr RN. 2010. Perception of landowners concerning conservation, grazing, fire and eastern redcedar management in tallgrass prairie. *Rangeland Ecology and Management* 63: 645-654.
- Nathan R, Muller-Landau HC. 2000. Spatial patterns of seed dispersal, their determinants and consequences for recruitment. *Trends in Ecology and Evolution* 15: 278-285.
- O'Connor TG. 1995. *Acacia karroo* invasion of grassland: environmental and biotic effects influencing seedling emergence and establishment. *Oecologia* 103: 214-223.
- O'Connor TG. 1996. Individual, population and community response of woody plants to browsing in African savannas. *Bulletin of the Grassland Society of southern Africa* 7, Supplement 1: 14-18.
- Or K, Ward D. 2004. The effects of seed quality and pipercolic and djenkolic acids on bruchid beetle infestation in water deficit-stressed *Acacia* trees. *Journal of Chemical Ecology* 30: 2291-2302.
- Palmer SCF, Hester AJ, Elston DA, Gordon IJ, Hartley SE. 2003. The perils of having tasty neighbors: grazing impacts of large herbivores at vegetation boundaries. *Ecology* 84: 2877-2890.
- Papachristou TG, Dziba LE, Provenza FD. 2005. Foraging ecology of goats and sheep on wooded rangelands. *Small Ruminant Research* 59: 141-156.

- Provenza FD, Villalba JJ, Dziba LE, Atwood SB, Banner RE. 2003. Linking herbivore experience, varied diets, and plant biochemical diversity. *Small Ruminant Research* 49: 257–74.
- Riginos C. 2009. Grass competition suppresses *Acacia* growth across multiple demographic stages. *Ecology* 90: 335-340.
- Robbins CT. 1993. *Wildlife Feeding and Nutrition*. (2<sup>nd</sup> ed). Academic Press, New York, USA.
- Rohner C, Ward D. 1997. Chemical and mechanical defense against herbivory in two sympatric species of desert *Acacia*. *Journal of Vegetation Science* 8: 717-726.
- Rohner C, Ward D. 1999. Large mammalian herbivores and the conservation of arid *Acacia* stands in the Middle East. *Conservation Biology* 13: 1162-1171.
- Salazar A. 2010. *Seed dynamics and seedling establishment of woody species in the tropical savannas of Central Brazil (Cerrado)*. Unpubl. PhD thesis, University of Miami, Florida, USA.
- Scogings PF, Mopipi K. 2008. Effects of water, grass and N on responses of *Acacia karroo* seedlings to early wet season simulated browsing: aboveground growth and biomass allocation. *Journal of Arid Environments* 72: 509-522.
- Shayo CM, Uden P. 1998. Recovery of seed of four African browse shrubs ingested by cattle, sheep and goats and the effect of ingestion, hot water and acid treatment on the viability of the seeds. *Tropical Grasslands* 32: 195-200.

- Shrader AM, Bell C, Bertolli L, Ward D. 2012. Forest or the tree: at what scale do elephants make foraging decisions? *Acta Oecologica* 42: 3-10.
- Titus CH, Provenza FD, Perevolotsky A, Silanikove N. 2000. Preference for foods varying in macronutrients and tannins by lambs supplemented with polyethylene glycol. *Journal of Animal Science* 78: 1443-1449.
- Trollope WSW. 1980. Controlling bush encroachment with fire in the savanna areas of South Africa. *Proceedings of the Grassland Society of southern Africa* 15: 173-177.
- van Auken OW. 2009. Causes and consequences of woody plant encroachment into western North American grassland. *Journal of Environmental Management* 90: 2931-2942.
- Vermeire LT, Mitchell RB, Fuhlendorf SD, Gillen RL. 2004. Patch burning effects on grazing distribution. *Journal of Range Management* 57: 248-252.
- Ward D. 2005. Do we understand the causes of bush encroachment in African savannas? *African Journal of Range and Forage Science* 22: 101-106.
- Ward D, Esler KJ. 2010. What are the effects of substrate and grass removal on recruitment of *Acacia mellifera* seedlings in a semi-arid environment? *Plant Ecology* 212: 245-250.
- Ward D, Wiegand, K, Getzin S. 2013. Walter's two-layer hypothesis revisited: back to the roots! *Oecologia* 172: 617-630.
- Ward D, Young TP. 2002. Effects of large mammalian herbivores and ant symbionts on condensed tannins of *Acacia drepanolobium* in Kenya. *Journal of Chemical Ecology* 28: 913-929.

Whitacre MK, Call CA. 2006. Recovery and germinability of native seed fed to cattle. *Western North American Naturalist* 66: 121-128.

Wigley BJ, Bond WJ, Hoffman MT. 2010. Thicket expansion in a South African savanna under divergent land use: local vs. global drivers? *Global Change Biology* 16: 964-976.