

**ENVIRONMENTALLY SUSTAINABLE GRASSHOPPER CONTROL IN AN
ECOLOGICALLY PROTECTED HABITAT**

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Abstract

Scientific literature indicates potential for using plant extracts to control arthropod pests thereby decreasing the amount of synthetic chemicals introduced into the ecosystem. The research presented below tested several control candidates in a field setting to determine if selected oils can be used to control grasshopper infestations. Two field studies tested the effects of five plant extract oils on grasshopper pests in southern Alberta: *Rosmarinus officinalis*, *Cedrus deodorata*, *Melaleuca alternifolia*, *Eucalyptus globulus*, and *Azadirachta indica*. Grasshopper abundance increased in the first study in all plots and decreased in the second study in all plots. A third study was conducted in a greenhouse where grasshoppers were treated with two concentrations of cedarwood and rosemary oil and were monitored for eight days for mortality and behavioural effects. A non-target study was conducted in order to determine if control candidates would negatively affect other beneficial arthropods. Cedarwood, neem oil and carbaryl bait were tested on the mortality of Carabidae and Phalangiidae using pitfall trap sampling.

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Table of Contents

| | |
|--|------------|
| Abstract | iii |
| Acknowledgements | iv |
| Chapter 1: General Introduction | 1 |
| Plant extracts and essential oils as potential pest controls..... | 5 |
| Objectives of the thesis and thesis structure | 14 |
| Literature Cited | 16 |
| Chapter 2: Field Experiments – testing plant extracts for effectiveness of grasshopper control in a field setting | 20 |
| Abstract..... | 20 |
| Introduction | 21 |
| Materials and Methods | 23 |
| Selection of Control Candidates | 23 |
| Experimental Design | 24 |
| Formulation and Application..... | 26 |
| Ecological Sampling and Statistical Analysis | 27 |
| Results..... | 28 |
| Effect – Long-plot Study | 28 |
| Effect – Field Study | 30 |
| Discussion | 32 |
| Conclusions..... | 34 |
| Literature Cited | 36 |
| Chapter 3: Cage experiments – testing of pesticidal effects of two essential oils in a controlled environment | 38 |
| Abstract..... | 38 |
| Introduction | 40 |
| Materials and Methods | 41 |
| Experimental Design | 42 |
| Formulation and Application..... | 43 |
| Monitoring and Statistical Analysis | 44 |
| Results..... | 44 |
| Discussion & Conclusions | 48 |
| Literature cited | 51 |
| Chapter 4. Non-target impacts – testing effects of grasshopper control candidates on two arthropod families: Carabidae (ground beetles) and Phalangiidae (daddy longlegs) | 52 |
| Abstract..... | 52 |
| Introduction | 54 |
| Materials and Methods | 55 |
| Selection of Control Candidates | 55 |

| | |
|--|-----------|
| Experimental Design | 56 |
| Formulation and Application..... | 58 |
| Ecological Sampling and Statistical Analysis | 59 |
| Results..... | 60 |
| Effect – Carabidae | 60 |
| Effect – Phalangidae | 65 |
| Discussion | 69 |
| Conclusions..... | 71 |
| Literature cited | 72 |
| Chapter 5: Using Geographic Information Systems to estimate grasshopper spatial distribution and infestation levels at Jefferson Community Pasture | 73 |
| Abstract..... | 73 |
| Introduction | 74 |
| Materials and Methods | 75 |
| Results..... | 77 |
| Discussion | 78 |
| Conclusions..... | 82 |
| Literature Cited | 88 |
| Chapter 6: Summary and Conclusions..... | 89 |
| Summary | 89 |
| Recommendations for further research | 91 |
| Recommendations for DUC/JCP grasshopper management plan | 93 |
| Conclusions..... | 96 |
| Literature cited | 98 |

List of Figures

| | |
|--|----|
| Figure 1.1 Location of Jefferson Community Pasture..... | 3 |
| Figure 1.2. Map provided by DUC showing sections of JCP and partnering organizations | 4 |
| Figure 2.1 Long-plot Study - Average grasshopper abundance of three blocks separated by treatment..... | 29 |
| Figure 2.2. Average grasshopper abundance of four blocks separated by treatment | 31 |
| Figure 3.1. Mean percent mortality per treatment with 95% confidence intervals | 45 |
| Figure 3.2. Average percent mortality from 5 blocks | 46 |
| Figure 3.3. Average moults per treatment showing standard error..... | 48 |
| Figure 4.1. Example of pitfall trap locations in 1 hectare treatment plot..... | 57 |
| Figure 4.2. Example of one block – each square represents one hectare treatment plot (5 traps)..... | 58 |
| Figure 4.3. Mean percent reduction of carabids from two weeks before treatment to two weeks after treatment for each treatment with 95% confidence intervals | 61 |
| Figure 4.4. Mean percent reduction of phalangiids from two weeks before treatment to two weeks after treatment for each treatment with 95% confidence intervals..... | 66 |
| Figure 5.1. Grasshopper density point counts grid for 2004. | 76 |
| Figure 5.7a. Rainfall in Cardston, AB for July 2003..... | 79 |
| Daily Total Rain for July 2004 | 79 |
| Figure 5.7b. Rainfall in Cardston, AB for July 2004 | 79 |
| Figure 5.8. Rainfall in Cardston, AB for August 2004..... | 80 |
| Figure 5.2. Kriged GIS map of grasshopper abundance in the study area; sampled points & lakes are overlain | 83 |
| Figure 5.3. Kriged GIS map of grasshopper abundance in the study area; lakes are overlain | 84 |
| Figure 5.4. Kriged GIS map of grasshopper abundance in the study area; lakes are overlain | 85 |
| Figure 5.5. Kriged GIS map of grasshopper abundance in the study area; lakes are overlain | 86 |
| Figure 5.6. Kriged GIS map of grasshopper abundance in the study area; lakes are overlain | 87 |

List of Tables

| | |
|--|----|
| Table 2.1. Oils selected for study | 24 |
| Table 2.2. Cost of oils per litre | 26 |
| Table 2.3. Long-plot Study - Percentage reduction in grasshopper abundance post treatment..... | 28 |
| Table 2.4. Summary of ANOVA statistics for effects of treatments on grasshopper abundance in Long-plot Study..... | 30 |
| Table 2.5. Field Study - Percentage reduction in grasshopper abundance post treatment | 30 |
| Table 2.6. Summary of ANOVA statistics for effects of treatments on grasshopper abundance in Field Study | 32 |
| Table 3.1. Summary of ANOVA statistics for effects of treatments on <i>M. packardii</i> mortality | 44 |
| Table 3.3. Summary of ANOVA statistics for effects of treatments on <i>M. packardii</i> moulting | 46 |
| Table 3.4. Descriptive Statistics – Average moults per treatment on day 3 | 47 |
| Table 4.1. Carabids per plot (5 traps per plot) collected from two weeks before to two weeks after treatment | 60 |
| Table 4.2. Summary of ANOVA statistics for treatment effects on carabid abundance for all pitfall traps | 61 |
| Table 4.3. Summary of ANCOVA statistics for treatment effects on carabid abundance for all pitfall traps | 62 |
| Table 4.4. Summary of ANCOVA statistics for treatment effects on carabid abundance for centre pitfall traps only..... | 63 |
| Table 4.5. Abbott’s adjusted percent mortality for carabids shown for each block | 64 |
| Table 4.6. Abbott’s adjusted percent mortality for carabids shown overall (blocks combined)..... | 64 |
| Table 4.7. Phalangiids per plot (5 traps per plot) collected from two weeks before to two weeks after treatment..... | 65 |
| Table 4.8. Summary of ANOVA statistics for treatment effects on phalangiid abundance for all pitfall traps | 66 |
| Table 4.9. Summary of ANCOVA statistics for treatment effects on phalangiid abundance for all pitfall traps | 67 |
| Table 4.10. Summary of ANCOVA statistics for treatment effects on phalangiid abundance for centre pitfall traps only..... | 67 |
| Table 4.11. Abbott’s adjusted percent mortality for phalangiids shown for each block..... | 68 |
| Table 4.12. Abbott’s adjusted percent mortality for phalangiids shown overall (blocks combined) | 68 |

Chapter 1: General Introduction

In the southwestern Prairie Provinces of Canada, especially in periods of drought, grasshopper infestations pose a significant economic threat to land managers. The insects are notorious for causing extensive crop damage because they multiply quickly, are difficult to kill and can cause significant damage in a very short period. After emergence, grasshopper pests are potentially damaging in all growth stages, and therefore present damage potential from late May to September (Olfert & Slinkard 1999). Olfert & Slinkard (1986a, in Olfert 1999) estimate grasshopper damage to cereal crops over the past 40-50 years to be an average annual loss of \$6 million, and up to \$200 million in an outbreak year. Johnson & Dolinski (1990) estimated over 400,000 litres of pesticides were applied in Alberta for grasshopper control in 1995 alone. In a survey of provincial and federal research, Johnson *et al.* (1996) estimated crop damage caused by grasshoppers from 1985-1991 to have cost \$253.5 million in Saskatchewan and \$73 million in Alberta. Because grasshopper control is economically costly for landowners and can be environmentally costly for non-target organisms, and thus the ecosystem, it is desirable to limit pesticide use to areas of high concentrations of pests and find control methods that are ecologically sustainable.

Ducks Unlimited Canada (DUC) is a national, private, non-profit organization that works to provide wetland and habitat conservation, restoration, and

management for North American waterfowl. DUC was formed in 1938 to support conservation efforts in Canada (DUC 2005). In the southern Prairies, approximately 70 percent of wetland habitat has been destroyed or disturbed over the last 100 years (DUC 2005). Habitat loss due to increase in agriculture and spread of urban areas has lead to a decrease in viable nesting grounds for the northern pintail, *Anas acuta* (USDOJ 1991). As a result, DUC has targeted wetland areas in southern Alberta and Saskatchewan that will provide suitable nesting areas for pintails. DUC works with landowners, government agencies and other non-profit agencies in order to establish these areas.

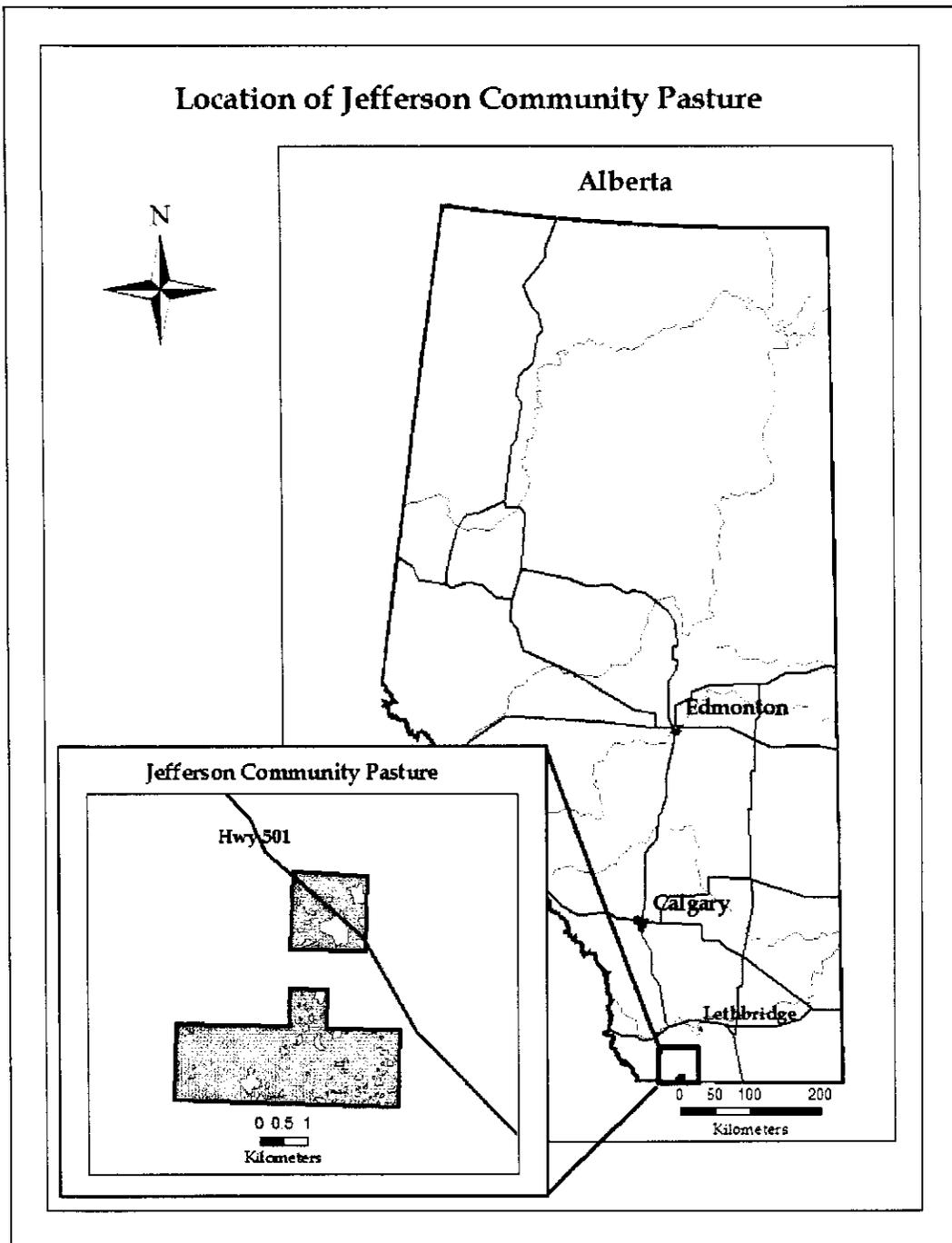


Figure 1.1 Location of Jefferson Community Pasture

In 2002, DUC purchased ~1200 hectares of land 15 km southeast of Cardston, AB (Figures 1.1 & 1.2) in order to provide suitable habitat for the northern pintail.

This site was named by DUC managers as Jefferson Community Pasture and will

be referred to as JCP in this paper. JCP was purchased in collaboration with the Nature Conservancy of Canada and Alberta Conservation Association in order to restore the ecological condition of the land and use it for long-term ecological monitoring (Figure 1.2). The land in this area is rich in pothole ponds in the spring and, when managed appropriately, will provide key habitat for the northern pintail. Incorporating land use practices that reduce intensive management of the land, such as converting cropland to forage, or fall seeding, will allow undisturbed spring nesting grounds necessary for sustaining pintail populations (DUC 2005).

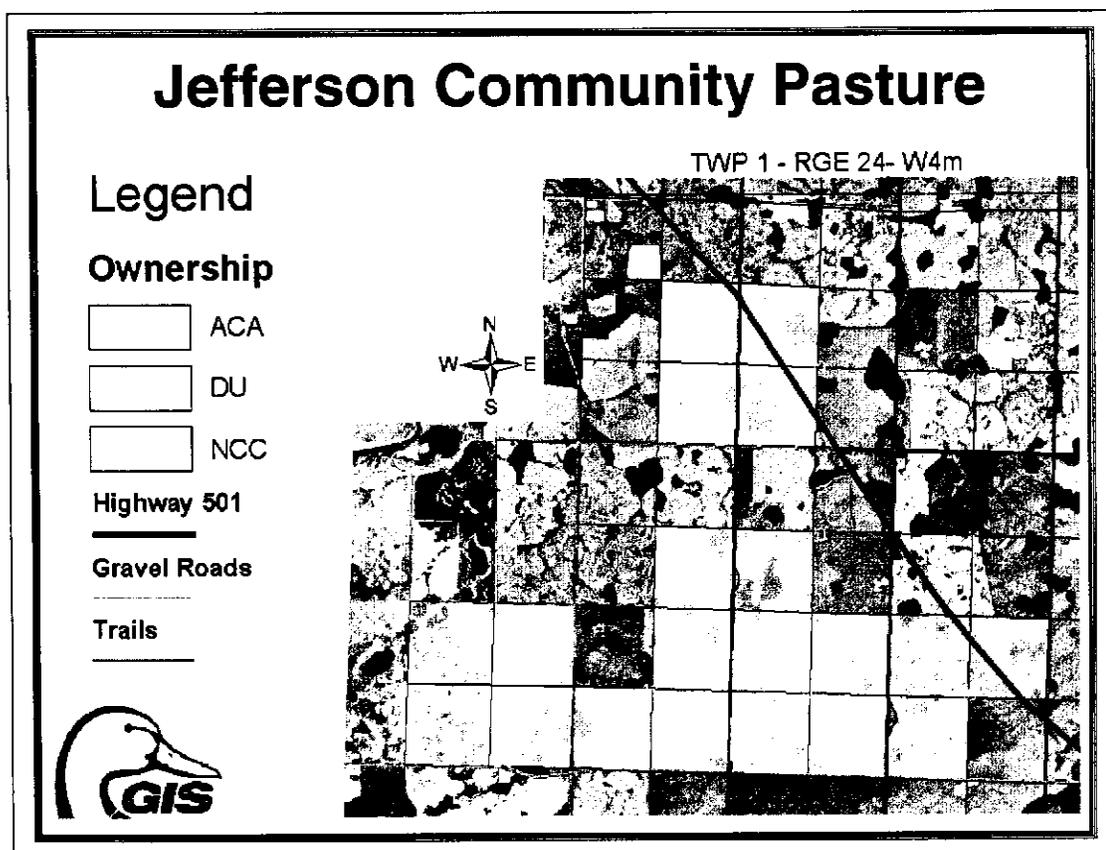


Figure 1.2. Map provided by DUC showing sections of JCP and partnering organizations

The DUC JCP site falls in a zone of moderate to severe infestations of grasshoppers (Orthoptera: Acrididae), the most significant and widespread pest of crops and pastures in western Canada (Johnson *et al.* 1986). Conservation efforts and increased environmental awareness have spurred the desire for insect pest management options that are less harmful to the environment than commonly used synthetic insecticides. Land managers, such as DUC, are investing in new ways to control insects that cause crop damage without posing a threat to the environment by destroying pollinators, poisoning organisms that rely on pest insects for food sources, or contaminating nearby water sources. Restrictions and prohibitions on pesticide spraying adopted by DUC as part of its sustainable management plan and commitment to general ecosystem health accentuate the problem of controlling the pest without putting the environment at risk.

Plant extracts and essential oils as potential pest controls

Plants produce toxins that are biologically active in repelling and killing insects, but are less persistent in the environment, and potentially less harmful to non-target organisms than conventional synthetic insecticides (Isman 2002).

According to Kostyukovsky *et al.* (2002), most terpenes and phenols found in plant essential oils, the biologically active extracts used to control pests, have minimal vertebrate toxicity and are USDA-approved as GRAS (Generally Regarded As Safe). Hence, extracting these compounds from plants as a method

of grasshopper pest control is a viable alternative to the use of synthetic chemicals.

Because of the peculiar nature of interspecific toxicity of different compounds (Isman 2000), this graduate research was designed to test a variety of essential oils to determine which of these might have an effect on grasshoppers. Literature search into the use of essential oils for controlling insect pests established that a wide variety of essential oils are being tested and found effective on many different insects in various stages of insect development. Control of insect pests using essential oils has been achieved in a range of settings including stored products, household application, gardening and agricultural use (Isman 2000). No published research regarding the effects of essential oils for controlling grasshoppers was found; therefore, oils were selected by reference to their effect on other insect species in published research, and by cost and availability.

Insecticidal activity of essential oils in stored product control has been documented against various insect species including Coleoptera: *Sitophilus oryzae* (Curculionidae), *Rhizopertha dominica* (Bostrichidae), *Tribolium castaneum* (Tenebrionidae), *Oryzaephilus surinamensis* (Silvanidae), *Trogoderma granarium* (Dermestidae), and Lepidoptera: *Ephestia cautella* (Phycitidae), *Plodia interpunctella* (Phycitidae) and the cotton bollworm moth, *Helicoverpa armigera* (Noctuidae), using essential oils from the family Labiatae (Kostyukovsky *et al.*

2002). Using essential oils extracted from anise, *Pimpinella anisum*, cumin, *Cuminum cyminum*, eucalyptus, *Eucalyptus camaldulensis*, oregano, *Origanum syriacum* var. *bevanii*, and rosemary, *Rosmarinus officinalis* Tunç *et al.* (2000) found extracts to be effective when used on the confused flour beetle, *Tribolium confusum* (Coleoptera: Tenebrionidae), and the Mediterranean flour moth, *Ephestia kuehniella* (Lepidoptera: Pyralidae). Huang *et al.* (2000) also reported success in stored product control of *Sitophilus zeamais* (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae) by using essential oils extracted from garlic oil. In conducting experiments to control the rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae), in stored product conditions Lee *et al.* (2001) found eucalyptus oil to be effective. Wang *et al.* (2001) found six plant essential oils to be effective against *Liposcelis bostrychophila* (Psocoptera, Liposcelididae); oils included *Citrus tangerina*, *Citrus aurantium*, *Citrus bergamia*, *Pinus sylvestris*, *Cupressus funebris*, and *Eucalyptus citriodora*.

In greenhouse, field and household settings where fumigant toxicity is not always applicable, Tunç & Sahinkay (1998) used cumin, *Cuminum cyminum*, extract and oregano, *Origanum syriacum* var. *bevanii*, to control infestations of the carmine spider mite, *Tetranychus cinnabarinus* (Acari: Tetranychidae), and the cotton aphid, *Aphis gossypii* (Hemiptera: Aphididae). Lee *et al.* (1997) tested 34 monoterpenoids from essential oil extracts on larva of western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) and found citronella oil

to be the most toxic. Peterson *et al.* (2002) found essential oil from catnip, *Nepetia cataria*, effective in repelling the German cockroach, *Blattella germanica* (Diptera: Blattellidae). Against the Hessian fly, *Mayetiola destructor* (Diptera: Cecidomyiidae), Lamiri *et al.* (2001) found *Mentha pulegium*, *Origanum compactum*, *Origanum majorana* to be the most toxic, after testing 19 essential oils. *Cinnamomum zeylanicum* and *Thymus spp.*, were most effective against fruit flies, *Ceratitis capitata* (Diptera: Tephritidae), when oil was sprayed on the insects' food and ingested (Sanna Passino *et al.* 1999). Ngoh *et al.* (1998) found that eugenol, methyleugenol, safrole, and isosafrole had contact and fumigant toxicity to adult American cockroaches, *Periplaneta americana* (Blattodea: Blattidae). Most effective against the codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae), was lavender, pennyroyal, rue, garlic and patchouli oil (Landolt *et al.* 1999). Ntiamoah *et al.* (1996) found pine oil a viable field application against the onion maggot, *Delia antiqua*, and that the oil acts as a deterrent for oviposition.

Research into mode of action by essential oils on insects has found a variety of possible explanations for how the essential oils affect their targets.

Kostyukovsky *et al.* (2002) found that, when used as fumigants for stored product insect control of selected beetle species (Coleoptera) and moth species (Lepidoptera), essential oils have a neurotoxic mode of action causing symptoms of hyperactivity, convulsions and tremors followed by paralysis ('knock down') – similar to insects' reaction to synthetic pesticides, such as pyrethroids. Sanna

Passino *et al.* (1999) reported that *Cinnamomum zeylanicum* and *Thymus spp.* had a depressive effect on the nervous system of fruit flies when they consumed food sprayed with essential oil extractions, and microscopic examination revealed anomalies in the gut region, when compared to control. In tests evaluating response to oil extracts of the twospotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae) and the house fly, *Musca domestica* (Diptera: Muscidae), Lee *et al.* (1997) suggested that the lipophilic property of most monoterpenoids found in essential oils may interfere with basic metabolic biochemical, physiological and behavioural functions of insects. In an experiment using essential oil from catnip, *Nepeta cataria*, as repellent for the German cockroach, antennectomised insects showed no response to concentrations that were active against intact insects (Peterson *et al.* 2002).

One possible explanation for the neurological effects of essential oils on insects offered by Kostyukovsky *et al.* (2002) is that the octopaminergic system, which plays a key role as a neurotransmitter, neurohormone and neuromodulator in invertebrate systems of insects, is disrupted by allelochemicals – mainly monoterpenes and phenols. Isman (2000) confirms this and states that lack of octopamine receptors in vertebrates may account for selectivity of essential oils as insecticides because they are toxic to insects but not to mammals. Decreased persistence in the environment and low toxicity to mammals makes essential oils desirable as pest control alternatives. Though much of the literature reports

effectiveness of oils in a field setting, few experiments have been conducted in a true field setting – most were conducted in greenhouses used to simulate a field setting. Therefore both greenhouse and field trials were used in this study.

The neem tree, *Azadirachta indica* (Meliaceae), has provided other plant extracts that have shown potential as alternatives to synthetic chemical pest control agents. Neem is native to India and is now grown in many countries in Asia and Africa, and in tropical areas of the New World (Lewis and Elvin-Lewis 1983 in Koul *et al.* 1990). Neem is known for its medicinal properties in India – it is used as antimalarial medication, an antiseptic, antimicrobial medication, bronchitis control, and as a healing agent against skin disorders and gum disease (Koul *et al.* 1990), attesting to its low toxicity to humans. Neem oil and other neem products have been used for many years in Africa and Asia to reduce grasshopper feeding on crops (Schmutterer 1990) and has been more recently studied for control of a variety of crop pests in Canada (Bomford & Isman 1996). Use of neem to control pests has been achieved in a number of different ways including insect growth regulation, disruption of metamorphosis, antifeedant effects, and deterrence of oviposition (Schmutterer 1990). Neem has shown some efficacy in controlling acridid pests, but its effect on the major grasshopper pests of the Prairies is largely unknown. One of the main grasshopper pest species in Alberta is the migratory grasshopper, *Melanoplus sanguinipes*, and although Isman (2002) states that the migratory grasshopper is completely insensitive to

neem, there is no other literature indicating to what extent neem affects this or other pest species.

There are a variety of applications of neem products for protection against stored grain pests and field pests. These include neem leaves (fresh or dried powder), leaf extract sprayed on vegetation, neem seed/kernel extract, neem kernel powder, de-oiled neem cake and neem oil. Each has shown some measure of efficacy against insect pests in either stored product or field situations (Prakash & Rao 1997). Of the plant extracts isolated from the neem tree, azadirachtin and salannin are reported to be the most toxic of the more than seventy limonoid-type terpenes isolated from neem (Bomford & Isman 1996; Govindachari *et al.* 2000). Isman *et al.* (1991) states that azadirachtin is the most potent natural insect antifeedant discovered to date. With regard to mode of action, Stark & Walter (1995) suggest that presence of neem oil increases efficacy of azadirachtin because it facilitates penetration of the insect cuticle.

Schmutterer (1990) states that neem derivatives have been effective in controlling insect pests in the following families: Orthoptera, Heteroptera, Homoptera, Hymenoptera, Coleoptera, Lepidoptera and Diptera. Examples of research into neem extracts that have provided control against pests are described below.

Isman *et al.* (1991) report neem being an effective control agent against the variegated cutworm, *Peridroma saucia* (Lepidoptera: Noctuidae), and the

European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae). Ulrichs *et al.* (2001) found that neem was an effective control against the cowpea aphid, *Aphis craccivora* (Hemiptera: Aphididae), and Gelbič & Němec (2001) found that neem caused restriction of reproduction in Egyptian cotton leaf worm, *Spodoptera littoralis* (Lepidoptera: Noctuidae). When tested alone and in combination with *Bacillus thuringiensis*, neem alone was lethal to both strains of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), and had synergistic effects on the Bt-R strain of beetles when combined with *B. thuringiensis* (Trisyono & Whalon 1999). Schmutterer *et al.* (1993) found neem to be a significant antifeedant for the desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae), and red locust, *Nomadacris septemfasciata* (Orthoptera: Acrididae) and the variegated grasshopper, *Zonocercus variegatus* (Orthoptera: Acrididae). Smirle & Wei (1996) found that topical application of neem on pear sawfly larvae resulted in reduced feeding, increased mortality and slower development. Joshi & Lockwood (2000) found aqueous extracts of neem leaves to have an antifeedant effect on *Oxya velox* (Orthoptera: Acrididae). Regarding non-insect pests, neem is reported to have nematicidal effects when neem plant by-products are incorporated into soil (Akhtar 2000).

Neem has been found to have an antifeedant effect on a variety of grasshopper and locust species (Schmutterer *et al.* 1993), but has not been studied extensively for its acridid control potential in Canada. Isman *et al.* (1991) tested neem on the

migratory grasshopper without promising results, however the two major pest species of grasshopper found at JCP are the two-striped grasshopper, *Melanoplus bivittatus* (Orthoptera: Acrididae), and the clear-winged grasshopper, *Camnula pellucida* (Orthoptera: Acrididae). Effects of neem on these grasshopper species were not reported in the literature.

As part of this graduate research, carbaryl bait was used in a non-target experiment to assess how the effects of neem and cedarwood oil compared to carbaryl bait and a control. Carbaryl bait is a grasshopper control measure that uses wheat bran treated with a carbamate pesticide. The bait is blown onto infested fields and insects with chewing mouthparts can ingest it. The benefits of using carbaryl bait relative to spray formulations are reduced rate of drift, lower concentrations of active ingredient, less residue build-up on crops and lower mammalian toxicity (Johnson & Henry 1987). Bran baits are safer for pollinators because of low drift rates and, generally, only insects that feed on carbaryl bait are killed by it (Gregory *et al.* 1992). The bran bait used in this study was Ecobait™, manufactured by Peacock Industries of Saskatoon, Saskatchewan. Non-target effects of essential oils, neem and, to some degree carbaryl bait, have not been widely studied, hence the desire to examine the difference in the effects of these treatments.

Objectives of the thesis and thesis structure

The first objective of this study was to determine whether selected plant extracts will reduce abundance and activity of grasshopper pests. Essential oils were selected based on past research, preliminary lab trials, availability and cost. Because research into effects of essential oils on grasshopper pests is novel, it was necessary to test several oils to determine which, if any, might increase mortality of these pest species. The second objective of this study was to investigate whether plant extract treatments and carbaryl-treated bran bait have a negative effect on non-target insect species. The third objective was to determine where the major grasshopper outbreaks occur on the DUC site, so that spatially/geographically selective treatment can be proposed, which also reduces the environmental impact of any type of pest control, be it organic or non-organic.

The experiments in Chapter 2 were designed to test a number of potential control candidates in two field situations (long-plot and field edge) and select oils that showed the highest level of effect on grasshopper mortality. In these two experiments, treatments were tested on relatively developed grasshopper populations containing mostly adults of the main pest species.

Oils that appeared to show the most potential in controlling grasshoppers were used in the experiment described in Chapter 3. The aim of this experiment was to

identify any potential effects of the two most effective oils in a more controlled environment, and to test them at two different rates on juvenile grasshoppers of a major pest species.

Chapter 4 includes a study intended to determine effects of neem and cedarwood oil on non-target insects, in comparison with a synthetic pesticide, carbaryl bait. Two families of non-target insects with similar feeding habits to orthopterans were chosen for this study – Carabidae (ground beetles) and Phalangidae (daddy longlegs).

Using GIS to estimate and map grasshopper densities has become a useful tool for land managers who wish to reduce unnecessary application of pesticides (Johnson *et al.* 1996). Chapter 5 includes maps of grasshopper densities in the summer of 2002 and 2003 and describes GIS as a tool for grasshopper management.

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Chapter 2: Field Experiments – testing plant extracts for effectiveness of grasshopper control in a field setting

Abstract

Conservation efforts and enhanced environmental awareness have increased the need for insect pest management options that are less harmful to the environment than commonly used insecticides. Research into plant extracts for use in insect control has been conducted on a large variety of essential oils and many species of insect pests. Results of previous studies indicate that there is great potential for using plant extracts to control pests, thereby decreasing the amount of synthetic chemicals introduced into the ecosystem and potentially reducing the known negative effects of pesticides in the environment.

Assessment of the effects of plant extracts on grasshoppers is sparse, therefore, this study is intended to test several control candidates in a field setting to determine if selected essential oils and neem can be used to control grasshopper infestations. Two studies are described here – one long-plot study and one field edge study – testing the effects of five plant extracts on grasshopper pests in southern Alberta. Grasshopper abundance increased in the long-plot study in all plots and decreased in the field edge study in all plots. Results of these studies found that, despite apparent effectiveness under laboratory conditions, oils had no significant effect on grasshopper abundance when compared to untreated control plots at operational field scales.

Introduction

Conservation efforts and enhanced environmental awareness have increased the desire for insect pest management options that are less harmful to the environment than commonly used insecticides. Land managers are searching for new ways to control pest insects without posing a threat to the environment, such as by destroying pollinators, poisoning organisms that rely on pest insects for food sources, or contaminating nearby water sources. In the southwestern Prairie provinces, especially in periods of drought, grasshoppers are extremely economically threatening to land managers (Olfert & Slinkard 1999). The insects are notorious for causing extensive crop damage because they are difficult to kill and, in large numbers, can cause significant damage in a very short period of time. Because grasshopper control is economically costly for landowners and can be environmentally costly for non-target organisms, it is desirable to limit pesticide use to areas of high concentrations of pests and find control methods that are ecologically and economically sustainable.

Plant extracts, including essential oils and neem, are known to have biologically active compounds that provide plants with natural pest control against phytophagous insects (Isman 1991; Lee *et al.* 1997). Because of their low toxicity to humans, essential oils provide possible alternatives to conventional, synthetic pesticides (Kostyukovsky *et al.* 2002). Similar to the effects of synthetic chemicals, essential oils can have a neurotoxic mode of action: symptoms include

hyperactivity, convulsions and tremors followed by paralysis, known as 'knock down' (Kostyukovsky *et al.* 2002). Additionally, some essential oils act as feeding deterrents (antifeedants) to some insect pests, deter insects from oviposition, reduce progeny, or delay growth and development (Kostyukovsky *et al.* 2002). Neem, *Azadirachta indica*, is a plant extract that has been used for many years in Africa and Asia for medicinal purposes and for insect control (Isman 1991). Isman (1991) states that azadirachtin, the active ingredient in neem, is the most potent natural insect antifeedant discovered to date. Schmutterer *et al.* (1993) and Joshi & Lockwood (2000) found neem to be an effective antifeedant for a variety of acridid species. Effects of neem on insects are similar to those of essential oil extracts: growth regulation, disruption of moulting, deterrence of oviposition and antifeedant effects (Schmutterer 1990). These extracts have been used by humans to control a variety of insect pests including stored product, household, greenhouse and field pests (Isman 2000). For this reason, a number of plant extracts were chosen for two field experiments to determine if any natural products used for control of other insects would work to reduce abundance and activity of invasive grasshopper populations.

Materials and Methods

Selection of Control Candidates

Essential oils were selected by evidence of effectiveness on other insect pests in previous studies, as well as by availability, and by cost. Table 2.1 shows the plant extracts selected for this study. Rosemary oil, *Rosmarinus officinalis*, is an expensive oil, however, different varieties of rosemary oil have been found to have an effect on insect species including *Cerattitis capitata* (Diptera) (Sanna Passano *et al.* 1999), on the confused flour beetle, *Tribolium confusum*, and the Mediterranean flour moth, *Ephestia kuehniella* (Tunç *et al.* 2000). Cedarwood oil, *Juniperus virginiana*, was found to be effective against the Formosan subterranean termite, *Coptotermes formosanus* (Zhu *et al.* 2001) and Anderson *et al.* (2002) found that cedar shaving water suspensions were repellent to fire ants, *Solenopsis invicta*. Cedarwood oil, *Cedrus deodorata*, was selected for this study because *Juniperus virginiana* was not available and *C. deodorata* was the cheapest cedarwood oil available. Eucalyptus oil was tested in a number of insect control experiments including Tunç *et al.* (2000) on the confused flour beetle and the Mediterranean flour moth, Tunç & Sahinkaya (1998) on the cotton aphid, *Aphis gossypii*, and Zhu *et al.* (2001) on the Formosan subterranean termite. Several different species of eucalyptus were used in previous research; *Eucalyptus globulus* was one variety used and the only variety available from our chosen supplier and was, therefore, selected for this research. Few journal articles report insecticidal activity of tea tree oil, *Melaleuca alternifolia*, but Walton *et al.* (2004)

found tea tree oil effective in reducing survival rates of the mite *Sarcoptes scabiei* var. *hominis*. Schmutterer (1990) found that neem increased insect mortality in Orthoptera, Heteroptera, Homoptera, Hymenoptera, Coleoptera, Lepidoptera and Diptera. Also, Schmutterer *et al.* (1993) found neem to be an effective antifeedant of the desert locust, *Schistocerca gregaria*, the red locust, *Nomadacris septemfasciata*, *Locusta migratoria migratorioides*, and the variegated grasshopper, *Zonocercus variegates*. Isman (2000 and 2002) reports differing responses of different pest species when exposed to essential oils and neem, therefore, a number of plant extracts were selected for this study to compare effects on grasshopper mortality in a field setting. Anecdotal information received from agriculturalists suggested that the addition of urea (liquid nitrogen 28-0-0) to neem would increase its effectiveness in the field, thus, this was also tested in this study.

Table 2.1. Oils selected for study

| | |
|------------|-------------------------------|
| Cedarwood | <i>Cedrus deodorata</i> |
| Eucalyptus | <i>Eucalyptus globulus</i> |
| Rosemary | <i>Rosmarinus officinalis</i> |
| Tea tree | <i>Melaleuca alternifolia</i> |
| Neem | <i>Azadirachta indica</i> |

Experimental Design

Two field experiments were conducted in the summer of 2003. The first experiment described here was conducted using long, narrow contiguous plots, end-to-end, in a cleared pathway surrounded by taller grass. The second

experiment was conducted near the field edge in larger plots. Treatments were applied at a Ducks Unlimited Canada property, Jefferson Community Pasture (JCP), approximately 15 km south of Cardston, Alberta (see Chapter 1).

Both studies used a randomised complete block design. The long-plot study consisted of three contiguous blocks with six treatments and an untreated control in each block. Plots were 100 m long and 6 m wide. For this experiment, an application rate of 250 ml of oil per hectare was used for each treatment. The field study was done in four consecutive blocks. Blocks were positioned along a field edge, approximately 30 m from the roadside. In each block, eight plots were established; plots measured 18 m by 28 m and were 2 m apart. One control plot in each block was chosen at random and received no treatment.

The treatments used in both experiments were commercially available plant extracts in the form of oils, with the exception of urea, which is available from farm suppliers as liquid nitrogen (28-0-0). Four of the plant extracts are sold for aromatherapy uses: cedarwood oil, eucalyptus oil, rosemary oil and tea tree oil (FPI Sales, Richmond, BC). The other plant extract, from the neem tree, *Azadirachta indica*, (active ingredient azadirachtin) was donated by Vgrove Inc. (Ontario). Each of the oil quantities, except neem, was calculated at a cost of \$30/ha (Table 2.2) based on an estimation of market rate for commonly used pesticides. The seven treatments (and amount of oil used) were cedarwood (1.3

L/ha), eucalyptus (1.5 L/ha), rosemary (0.75 L/ha), tea tree (1.0 L/ha), neem (1.2 L/ha), urea (1.2 L/ha), neem + urea (0.6 L/ha; combined 1.2L/ha), and an untreated control, on which water without extracts was applied at the same rate as for the treatments. Twice the recommended application rate of neem (1.2L/ha) was used, to be relatively consistent with the quantities of the other treatment formulations.

Table 2.2. Cost of oils per litre

| Product | Cost per litre (\$) | Rate @ \$30 / ha (L) |
|----------------|----------------------------|-----------------------------|
| Cedarwood | 23.00 | 1.3 |
| Eucalyptus | 20.00 | 1.5 |
| Rosemary | 40.00 | 0.75 |
| Tea tree | 30.00 | 1.0 |
| Neem | 30.00 | n/a |

Formulation and Application

Treatments for both experiments were mixed with water and an emulsifier (Sunlight™ dish soap) for total application rate of 80 L/ha. Approximately 2 ml of soap was used in each 80-litre application. Oils were emulsified in soap and water mixture by manually agitating sprayer tank and by tank motion during application. Application of treatments for each study were conducted on the same day: long-plot treatments were applied between 1600 and 1800 hours, July 3, 2003. Field treatments were applied between 0600 and 1000 hours, July 16, 2003. Both areas were treated using a Spray-Tech Systems Ltd. ATV 1810 quad-mounted sprayer with a 3 m boom. Spray jets were 50 cm from the ground and drift was observed to be minimal; wind speed was <15 km/h.

The land used for spray trials was seeded in the spring with mixed grasses (Ecobait™). This mix consists of meadow brome, intermediate wheat, pubescent wheat, tall fescue and alfalfa. Vegetation had grown to a height low enough that it did not interfere with the sprayer boom. Light rain fell on the morning of the field treatment, but stopped before application of treatment to the first plot was completed (tea tree oil, Block 1). Wind speed remained <15 km/h throughout treatment and air temperature ranged from 15 to 25°C during time of application.

Ecological Sampling and Statistical Analysis

Species and age composition of grasshoppers in the treatment areas were determined by sweep sampling and by observations made over the course of establishing the experiment locations. In the long-plot experiment, densities were estimated per m² by walking through the plots measuring density in five sections in each plot. Counts were made by two observers (DJ and MM). Density observations were made directly prior to application and 3 days after application. For the field experiment, densities were estimated using 0.25 m² sample quadrats. Ten sampling quadrats were placed in each treatment plot during the before-treatment count (320 quadrats in total) and remained in position throughout the course of the experiment. Quadrats were placed in highly vegetated areas where grasshoppers were most abundant in order to be able to detect any change in abundance before and after treatment. The number

of grasshoppers in each sampling quadrat was observed and recorded 1 day before treatment application and 1 and 3 days after application. Counts were made by three observers (DJ, MM and BT). Data were analysed using analysis of variance of percent reduction in number of grasshoppers.

Results

Effect – Long-plot Study

The long-plot study area consisted of approximately 80% clear-winged grasshopper, *Camnula pellucida* (Scudder) – the second most common grasshopper species at the JCP site. Grasshopper abundance in the plots before application was estimated to range from 12-100 grasshoppers per m². Age structure at the time of spraying ranged from juvenile to adult with the most dominant age range being 3rd to 5th instar.

Table 2.3 shows the average percent reduction of grasshopper abundance per m² in the long-plot study.

Table 2.3. Long-plot Study - Percentage reduction in grasshopper abundance post treatment

| <u>Treatment</u> | <u>% Reduction</u> |
|------------------|--------------------|
| Neem | -92.5 |
| Cedarwood | -96.0 |
| Rosemary | -121.1 |
| Untreated | -135.4 |
| Tea tree | -137.8 |
| Urea | -142.5 |
| Eucalyptus | -156.1 |

Grasshopper abundance increased for all treatments in the long-plot experiment. The lowest increase in abundance was in the plots treated with neem oil and, secondly, cedarwood oil: increases of 92 and 96%, respectively. Plots treated with eucalyptus oil, tea tree oil and urea increased in abundance more than the control: all increasing more than two-fold. Figure 2.1 shows the average increase in abundance at 3 days post treatment.

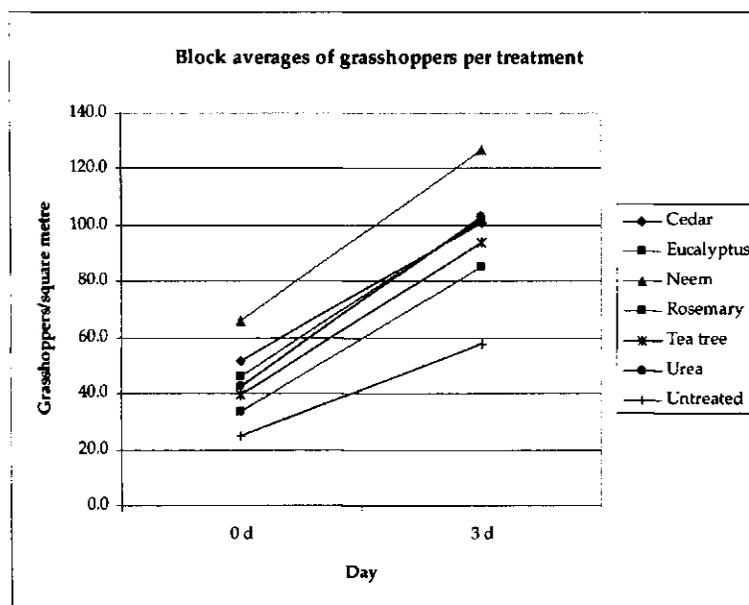


Figure 2.1 Long-plot Study - Average grasshopper abundance of three blocks separated by treatment

Results of the analysis of variance test (Table 2.4) showed no significant effect of treatments on grasshopper abundance, $F_{(6,12)} = 0.34$, $p = 0.91$. Percent reduction data were normally distributed and did not require transformation for analysis.

Table 2.4. Summary of ANOVA statistics for effects of treatments on grasshopper abundance in Long-plot Study

| Source of variation | df | MS | F | P |
|---------------------|----|----------|-------|-------|
| Block | 2 | 2631.32 | 0.084 | 0.920 |
| Treatment | 6 | 10542.42 | 0.336 | 0.905 |
| Residual | 12 | 31383.88 | | |

Effect – Field Study

In the area of the field study, the two-striped grasshopper, *Melanoplus bivittatus* (Say), was the most common grasshopper species; this species represents approximately 95% of all grasshoppers found at this site. Grasshopper abundance in the plots ranged from 12 to 72 grasshoppers per m² 1 day before treatment. Age structure at the time of spraying was approximately 60-80% adult, with the remaining 20-40% consisting of mainly 3rd to 5th instars.

Table 2.5 shows the average percent reduction of grasshopper abundance per m² in the field study.

Table 2.5. Field Study - Percentage reduction in grasshopper abundance post treatment

| Treatment | % Reduction | |
|------------|-------------|------|
| | 1 d | 3 d |
| Cedarwood | 7.4 | 33.1 |
| Eucalyptus | -8.6 | 37.3 |
| Neem | 22.4 | 55.8 |
| Neem/Urea | 21.6 | 55.2 |
| Rosemary | 14.0 | 36.7 |
| Tea tree | 28.3 | 62.1 |
| Urea | 20.6 | 53.1 |
| Untreated | 17.2 | 48.2 |

Grasshopper abundance decreased post treatment in all treated areas, with the exception of eucalyptus after 1 d, which increased in abundance by 8.6%, but decreased after 3 d by 37.3%. An average of grasshopper abundance per block is shown in Figure 2.2. A lower average percent reduction is observed for 1 d than 3 d post treatment. Untreated plots in both 1 and 3 d post treatment also show an increase in percent reduction.

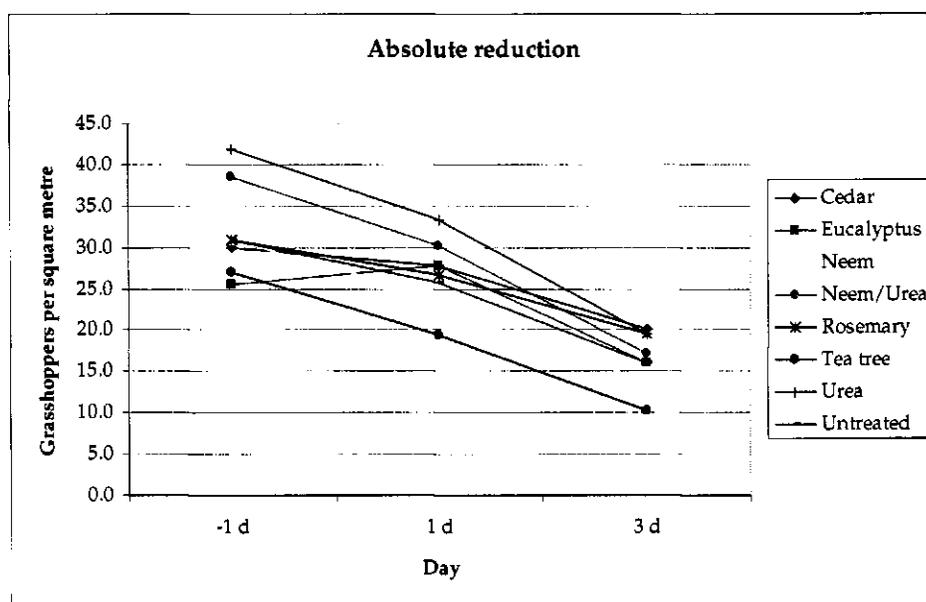


Figure 2.2. Average grasshopper abundance of four blocks separated by treatment

Results of the analysis of variance test (Table 2.6) show no significant effect of treatments on grasshopper abundance, $F_{(7,21)} = 2.16, p = 0.08$. Percent reduction data were normally distributed and did not require transformation for analysis.

Table 2.6. Summary of ANOVA statistics for effects of treatments on grasshopper abundance in Field Study

| Source of variation | df | MS | F | P |
|----------------------------|-----------|-----------|----------|----------|
| Block | 3 | 591.61 | 1.697 | 0.198 |
| Treatment | 7 | 754.29 | 2.164 | 0.081 |
| Residual | 21 | 348.55 | | |

Unlike the long-plot study, in the field study grasshopper abundance was more evenly spread in each of the plots before treatment and does not show a discernible decrease in abundance after treatment specifically for areas that began with higher densities.

Discussion

The long-plot study showed an increase in grasshopper abundance for all of the plots, treated or untreated, and no significant difference between any of the treatments. Similarly, no single treatment in the field study significantly decreased grasshopper abundance at a higher rate than that of the untreated plot. Overall, grasshopper abundance decreased for all plots in the field study, treated or untreated, however treatment had no significant effect on grasshopper abundance. Several explanations for these results are considered here.

First, because effects of essential oils as grasshopper control candidates have not been previously reported, no recommended rates were available. Therefore, the application rates for control candidates were selected not based on effectiveness shown in previous experiments, but based on approximate market cost for

commonly used pesticides. These rates may be too low to show significant adverse effects on grasshopper mortality. The rates applied in the long-plot study were significantly lower than that of the field study – this may partially account for the increase in grasshopper abundance in the long-plot study. However, the experimental design of the long-plot study may account for the increase in grasshopper density post treatment. The long-plot study was designed to determine if treatments would deter grasshoppers from dispersing into this area. For this reason, plots were end-to-end and only 6 m in length. Migration from grasshoppers in the untreated area adjacent to the treatment area may have moved in due to the shorter grass in the treatment area. This was not the case in the field study because plots were not directly adjacent to one another and vegetation cover did not vary between the treated to untreated areas.

Second, juvenile grasshoppers tend to be more sensitive to contact control methods due to their developing cuticles, increased sensitivity during development, and lesser ability to escape exposure because they cannot fly (Romoser & Stoffolano Jr. 1998). Age structure of grasshoppers in the field treatment area was approximately 60-80% adult at the time of spraying, with the majority of juvenile grasshoppers being in the later stages of development (3rd to 5th instar). These more developed insects may be more resistant to treatment (Romoser & Stoffolano Jr. 1998). As azadirachtin is a growth inhibitor (Isman *et*

al. 1991), the neem treatment may have been more effective, too, had the insects been in an earlier stage of development.

Third, many of the essential oils tested here are volatile substances and may, therefore, be affected by higher temperatures – they may evaporate more rapidly with increasing temperatures. Negative effects of oils on grasshopper survival could increase if candidates were applied during cooler periods, for example spraying treatments at dawn, before diurnal temperatures begin to rise.

Additionally, Zhu *et al.* (2001) state that there is an inverse relationship between bioactivity (effectiveness) and volatility of essential oils, suggesting that the more volatile substances may not be effective insect control candidates, especially in a field setting. However, Tunç & Sahinkaya (1998) found that certain greenhouse pests were affected by the plant extract vapours, suggesting that not only the liquid phase of plant extracts are effective in treating insect pests, but also the gas phase. In a field condition plant extract vapours are not contained in the environment such as in the case of greenhouses, but instead can be transported away from the area of treatment by wind and, therefore, lose effectiveness.

Conclusions

As none of the treatments used in the field experiments described in this chapter have been previously documented for effects on grasshopper mortality, there is vast potential for variation of experimental parameters, such as increasing

application rates, altering formulations, and spraying at lower temperatures. Microencapsulation could allow the more volatile substances longer persistence so that they do not evaporate as quickly and would be released on contact rather than when they were sprayed into the air. Further study into timing of application, effects on insects at different developmental stages and effects of temperature on evaporation rates may be needed to determine if these substances could prove useful in a field setting. The results of this study should not rule out the possibility of developing plant extracts for grasshopper control.

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Chapter 3: Cage experiments – testing of pesticidal effects of two essential oils in a controlled environment

Abstract

Certain essential oils are known to have antifeedant and insecticidal properties on a variety of insect pests. This experiment was designed to test the effect of two essential oils, cedarwood and rosemary oil, on the mortality, development and feeding habits of the grasshopper pest species *Melanoplus packardii*, one of the four major grasshopper pest species in southern Alberta. Two treatment rates were tested for each oil against an untreated control: a high and low concentration of cedarwood and a high and low concentration of rosemary. Rates for the low concentrations were calculated based on an economically feasible assigned value of ~\$30/ha. Cedar treatments were tested at concentrations of 1.3 L/ha and 5.2L/ha (4x low) and rosemary at concentrations of 0.75L/ha and 3.0L/ha (4x low). Tests were done in a controlled greenhouse environment using cages with sufficient wheat grown to sustain ~26 grasshoppers per cage for 2 weeks. Five replications were randomised in block design; 25 cages were used, each containing 21-25 grasshoppers (576 grasshoppers in total). Wheat, soil and grasshoppers were spray-treated simultaneously on day 0, and grasshoppers were monitored on day 0, 1, 2, 3, 5 and 8 days after treatment. Living and dead grasshoppers and moults were counted and feeding behaviour was assessed by monitoring the amount of wheat that had sustained signs of feeding damage. Although total mortality in the cedar high treatment was substantially higher

than in the other treatments (10% vs. 3-7%), there was large variability in samples. Results of the ANOVA test found that the effect of treatments on grasshoppers was not statistically significant ($F_{(4,4)} = 1.10, p = 0.39$). Mortality was not significantly affected by any of the treatments. Moulting and feeding behaviour were also observed and are discussed in this chapter.

Introduction

In an effort to find alternatives to conventional pesticides, research into the effectiveness of essential oils extracted from aromatic plants has been extensively studied. The desire to reduce use of synthetic pesticides stems from a variety of potentially harmful effects caused by their application including: negative effects on beneficial organisms, persistence in the environment, potential to contaminate water sources and development of insect resistance to these chemicals. Isman (2000) supports the development of insect control methods from essential oils because many essential oils demonstrate knockdown effects on various insect pests similar to that of conventional pesticides, but residues from these oils do not persist in the environment to the degree that synthetic pesticides do. Effects of essential oils as pest control for many insect pests have been documented; however, research into their effects on grasshopper pests has not been reported to date.

Kostyukovsky *et al.* (2002) suggests that a possible target for mode of action in essential oils is the octopaminergic neurotransmitter system in insects. According to Isman (2000), lack of octopamine receptors in vertebrates makes essential oils a potentially viable alternative to conventional pesticides because they pose a lesser threat to mammals. Also, because different insect species respond very differently to constituents of essential oils, the effects of a selected oil on one species cannot predict how another will be affected given the interspecific

toxicity of essential oils reported in the literature (e.g. Isman 2000). For this reason we selected two plant extracts that were reported to negatively affect insect pests and tested them at different concentrations to determine their effects on grasshoppers. In a previous study using rosemary oil extract, Sanna Passino *et al.* (1999) found that by spraying oil formulations onto food that was ingested by adult fruit flies, *Ceratitis capitata*, the oils caused a depressive effect on the nervous system and anomalies in the gut region when compared to that of the controls. Zhu *et al.* (2001) tested seven essential oils on the feeding behaviour of the Formosan subterranean termite, *Coptotermes formosanus*, and found that cedarwood oil was highly repellent. In laboratory trials, Anderson *et al.* (2002) found cedar shaving water suspensions were repellent to fire ant colonies, *Solenopsis invicta*.

The intention of this study was to investigate the effects of two essential oil extracts, cedarwood oil, *Cedrus deodorata*, and rosemary oil, *Rosmarinus officinalis*, on grasshoppers to determine whether either showed potential as antifeedant or insecticidal control of grasshopper pests.

Materials and Methods

Treatment candidates for this experiment were selected based on review of current literature on plant extracts, preliminary lab tests, and previous preliminary field tests. Cedarwood and rosemary oil were purchased from FPI

Sales, Richmond, BC. Grasshoppers used in this study were collected by sweep sampling on farmland ~5 km north of Coaldale, AB, and stored in cages at approximately 20°C in an insect-rearing laboratory at the Lethbridge Research Centre (LRC), Lethbridge, AB.

Experimental Design

We tested the effect of cedarwood and rosemary oil on the mortality, development and feeding habits of the grasshopper pest species *Melanoplus packardii*, one of the four major grasshopper pest species in southern Alberta. Cedarwood and rosemary oil were selected from the seven previously field-tested oils for use in greenhouse cage trials in order to observe insect behaviour under controlled environmental conditions. Five replications were randomised in block design; 25 cages were used, each containing 21-25 grasshoppers (576 grasshoppers in total). Grasshoppers were collected and sorted by species and stage of development. We collected ~700 Packard's grasshoppers, *M. packardii*, ranging from third to fifth instar. Two treatment rates were tested for each oil against an untreated control: a high and low concentration of cedarwood and a high and low concentration of rosemary. Rates for the low concentrations were calculated based on an economically feasible assigned value of ~\$30/ha. Cedar low treatments were at a concentration of 1.3 L/ha and 5.2L/ha (4x low) and rosemary at a concentration of 0.75L/ha and 3.0L/ha (4x low). Wheat, soil and

grasshoppers were spray-treated simultaneously on day 0, and grasshoppers were monitored on day 0, 1, 2, 3, 5 and 8 days after treatment.

Formulation and Application

Twenty-five grasshoppers were put in sterile metal pans (22 x 15 x 6 cm) and contained by mesh lids that enabled exposure to spray without escaping containment. Wheat was grown from seed for 12 days in 55 by 40 cm trays filled with 6 cm of soil. Grasshoppers and wheat trays were sprayed simultaneously for each corresponding treatment type and rate. Application of all treatments was conducted on June 21, 2004, between 0900 and 1100 using a Rogers sprayer with a 3 m boom. Treatments were mixed with water and an emulsifier (Sunlight™ dish soap) for an application rate of 80 l/ha. Approximately 2 ml of soap was used in each 80-litre application. Oils were emulsified in soap and water mixture by manually agitating spray bottles prior to treatment. Spray jets were 50 cm from the ground and minimal drift was observed, as winds were <15 km/h. Spraying was conducted in a field at LRC. Directly after spraying, wheat trays and grasshoppers receiving the same rate of treatment were put into mesh cages (55 x 40 x 9 cm) in a greenhouse at LRC for observation. Prior to treatment, the soil in the wheat trays was thoroughly soaked to ensure enough moisture for sustained growth of the wheat for the duration of the experiment.

Monitoring and Statistical Analysis

Grasshopper mortality, feeding behaviour and number of moults were observed and recorded on day 0, 1, 2, 3, 5 and 8. Effects were monitored in a controlled greenhouse environment using cages with sufficient wheat grown to sustain ~26 grasshoppers per cage for 2 weeks. Feeding behaviour was assessed by monitoring the amount of wheat that had sustained signs of feeding damage. Greenhouse temperatures were set at 25°C during the day (16 h) and 16°C during the night (8 h). Data were analysed using analysis of variance of percent reduction of grasshoppers. Additionally, we compared number of moults per treatment and observed feeding behaviour.

Results

Mean mortality after 8 days in the cedarwood high treatment appears significantly higher than in the other treatments (10% vs. 3-7%). Figure 3.1 shows the mean percent mortality per treatment and the large confidence intervals that result from large variability in samples. Results of the ANOVA test (Table 3.1) found that the effect of treatments on grasshoppers was not statistically significant, $F_{(4,4)} = 1.10$, $p = 0.39$.

Table 3.1. Summary of ANOVA statistics for effects of treatments on *M. packardii* mortality

| Source of variation | df | MS | F | P |
|---------------------|----|--------|-------|-------|
| Block | 4 | 37.306 | 1.325 | 0.303 |
| Treatment | 4 | 30.946 | 1.099 | 0.391 |
| Residual | 16 | 28.146 | | |

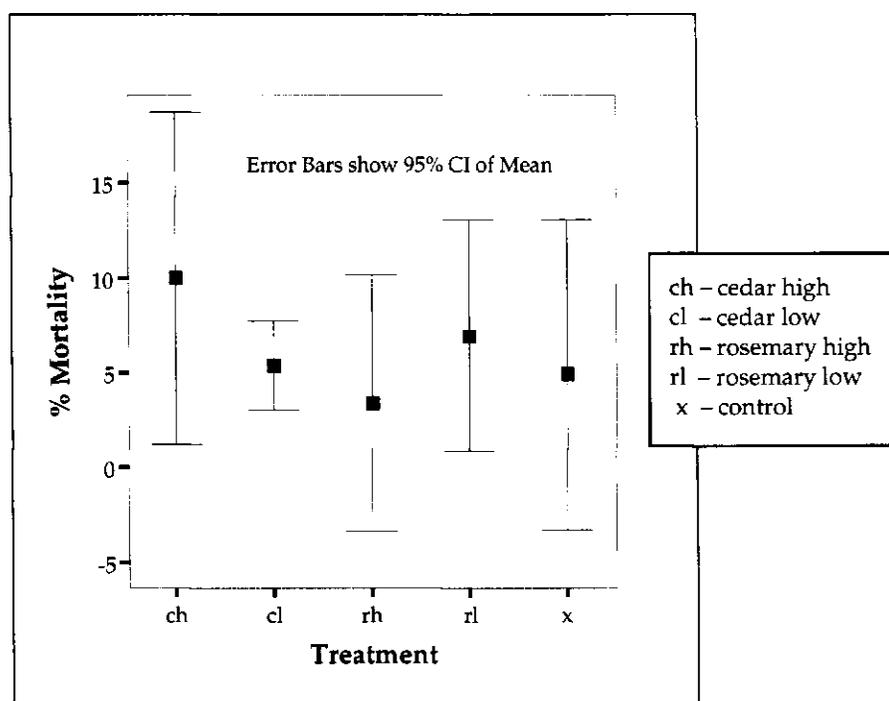


Figure 3.1. Mean percent mortality per treatment with 95% confidence intervals

As seen in Figure 3.2, percent mortality increases most in the group receiving the higher concentration of cedarwood oil, although percent mortality increases across all treatments and the control. The higher concentration of rosemary oil shows the lowest mortality.

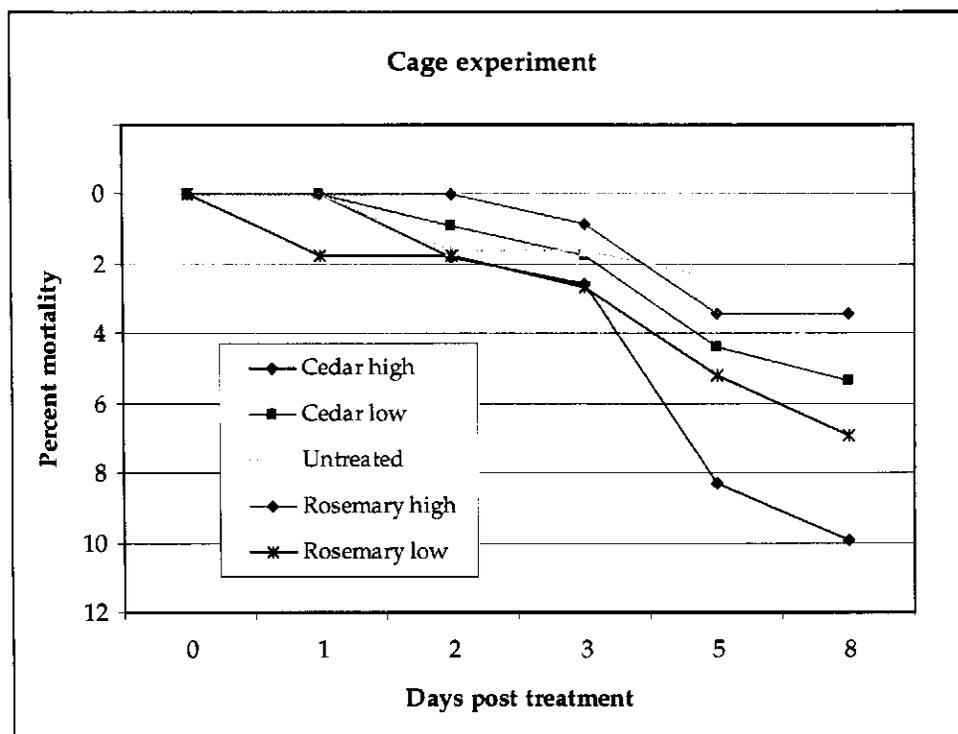


Figure 3.2. Average percent mortality from 5 blocks

Moulted grasshoppers were counted in each cage in days 1 and 3; no moulting was observed after day 3. Between 1 and 8 moulted grasshoppers were found in the cages on day 3, and on average 2.4 grasshoppers moulted in the cedar high treatment, 3.4 in the cedar low, 3.8 in the rosemary low, 4.6 in the control and 5.0 in the rosemary high.

Table 3.3. Summary of ANOVA statistics for effects of treatments on *M. packardii* moulting

| Source of variation | df | MS | F | P |
|---------------------|----|-------|-------|-------|
| Block | 4 | 3.840 | 1.497 | 0.250 |
| Treatment | 4 | 5.240 | 2.043 | 0.137 |
| Residual | 16 | 2.565 | | |

Based on the results of the ANOVA (Table 3.3), there is no significant effect of essential oil treatments on moulting behaviour, $F_{(4,4)} = 2.04, p = 0.14$.

Table 3.4. Descriptive Statistics – Average moults per treatment on day 3

| Treatment | N | Minimum | Maximum | Mean | | Variance |
|---------------|-----------|-----------|-----------|-----------|------------|-----------|
| | Statistic | Statistic | Statistic | Statistic | Std. Error | Statistic |
| Cedar high | 5 | 1.0 | 5.0 | 2.4 | .68 | 2.3 |
| Cedar low | 5 | 2.0 | 5.0 | 3.4 | .68 | 2.3 |
| Rosemary high | 5 | 2.0 | 8.0 | 5.0 | 1.00 | 5.0 |
| Rosemary low | 5 | 2.0 | 5.0 | 3.8 | .58 | 1.7 |
| Untreated | 5 | 3.0 | 7.0 | 4.6 | .75 | 2.8 |

Table 3.4 shows statistics for moulting on day 3 in each treatment. The higher concentration of rosemary oil had the most grasshoppers moulting with an average of 5.0 moults, while the higher concentration of cedarwood oil had the fewest number of grasshoppers moulting with an average of 2.4 moults. The mean number of moults for the control group is slightly lower (4.6) than that of the high concentration of rosemary oil (5.0). These data and their large variability are shown in Figure 3.3.

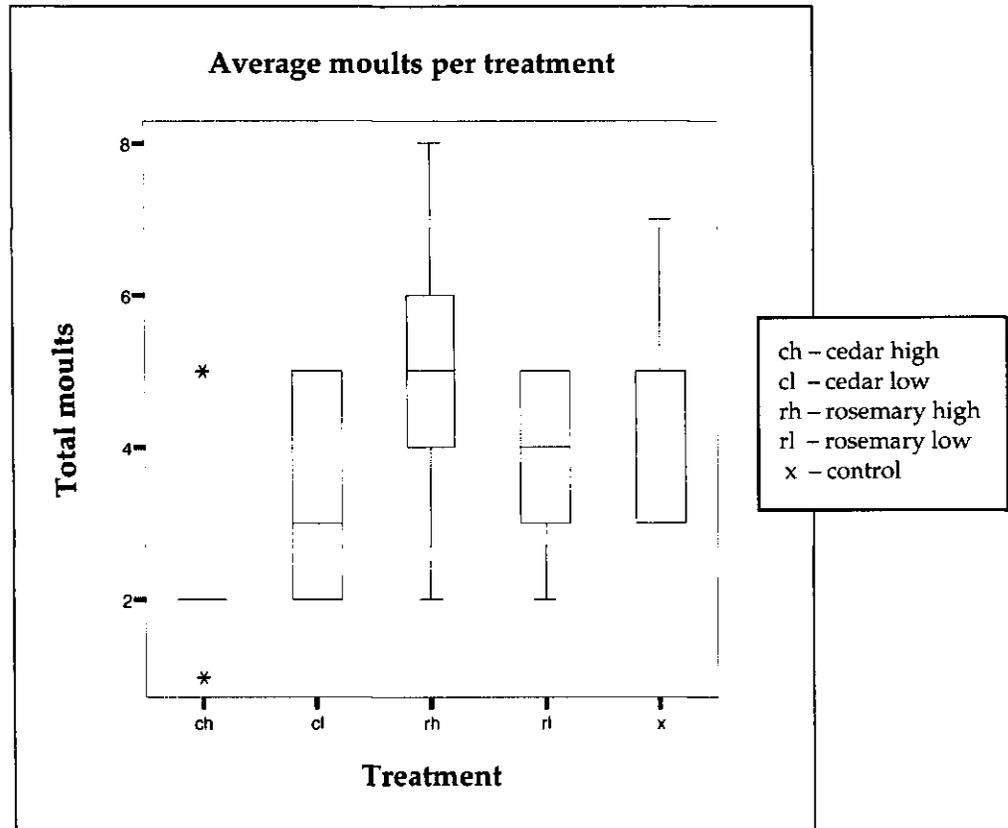


Figure 3.3. Average moults per treatment showing standard error

Feeding was reduced in all treatments compared to control groups for the first two days after treatment, but normal feeding resumed on the third day.

However, no quantitative data were collected and hence no statistical analysis has been performed.

Discussion & Conclusions

Although mean mortality in the cedar high treatment was higher than in the other treatments (10% vs. 3-7%), the variability within the samples was so great that the effect of treatments on grasshopper mortality was not statistically

significant. Thus, mortality was not significantly affected by any of the treatments. As the concentrations of control candidates were based on a rate calculated by economic viability, the effects of the selected oils is not definitive. The increase in grasshopper mortality for the high concentration of cedarwood oil shows potential for further investigation using higher application rates. Wheat growth did not appear to be negatively affected by the application of essential oils, suggesting that increasing the amount of oil in the treatment formulations is feasible without causing damage to vegetation. Additionally, because essential oils are volatile substances, searching for formulations or application techniques that allow the oils to evaporate more slowly may enhance their overall effect. Zhu *et al.* (2001) tested seven insect-active essential oils including cedarwood oil and states that bioactivity of essential oils is inversely related to volatility, therefore, bioactivity may be increased by adopting better methods of application, such as microencapsulation, that allow oils to be released upon contact, rather than having these rapidly evaporate during spraying.

Moulting effects, though not statistically significant, showed some variation per treatment. Again, this could be a result of concentrations of oils being too low, or a result of volatility and rapid evaporation of active ingredients in grasshopper control candidates. Grasshoppers exposed to the higher concentration of cedarwood oil moulted the least, therefore, there may be potential for causing disruption of development if cedarwood oil were applied at a higher rate. Also, if

oils were released upon contact with insect pests or on contact with vegetation, the potential for the toxins to enter the insect integument or be consumed would be greater, thereby enabling an increased probability of pest control effectiveness. This may not only hold true for increased mortality, but also for effect on feeding behaviour. And, because feeding behaviour was not quantified in this study, an experiment looking at amount of vegetation consumed after treatment may find feeding reduction significant enough that certain essential oils could prove to be a deterrent for grasshopper pests.

Isman (2000) found that essential oils demonstrate interspecific toxicity when tested on a variety of insect species. As treatments were tested on only one pest species of grasshopper, *M. packardii*, this study does not rule out the possibility that rosemary or cedarwood oil may negatively affect other grasshopper pest species. Though there are four grasshopper species in southern Alberta that pose the most significant economic threat to landowners, different densities of particular species occur in different areas. Therefore, a larger study testing the same essential oils on other pest species of grasshoppers may have a more significant effect.

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Chapter 4. Non-target impacts – testing effects of grasshopper control candidates on two arthropod families: Carabidae (ground beetles) and Phalangiidae (daddy longlegs)

Abstract

Plant extracts known to have antifeedant and insecticidal properties on a variety of insect pests were tested to determine if they have potential as alternative grasshopper control measures. In order to determine if they have negative effects on non-target organisms, two plant extracts, cedarwood and neem oil, and a conventional pesticide, carbaryl bait, were tested for effect on the mortality of two arthropod families – Carabidae (ground beetles) and Phalangiidae (daddy longlegs). One treatment rate was tested for each control candidate compared against an untreated control. Rates for the oil concentrations were selected based on application rates used in the studies discussed in the previous chapters. Cedarwood and neem oil were applied at concentrations of 1.3 L/ha and 1.2 L/ha, respectively. Carbaryl bait (Ecobait™) was applied at a standard market rate for control of grasshopper pests, at 4 kg/ha (80 g AI/ha). Experiments were conducted at Jefferson Community Pasture, a pasture owned by Ducks Unlimited Canada, located approximately 15 km south of Cardston, AB. Pitfall trap sampling was used to determine if carabid or phalangiid numbers decreased after treatment. Traps were emptied from two weeks before to two weeks after treatment. Results of ANCOVA tests showed no significant effect of any of the

treatments on carabids ($F_{(3,138)} = 0.54, p = 0.67$) or phalangiids ($F_{(3,140)} = 1.37, p = 0.31$) when compared to that of the controls.

Introduction

Use of synthetic pesticides can damage ecosystem health in ways such as negatively affecting beneficial organisms, persistence in the environment, potential to contaminate water sources, and development of insect resistance to these chemicals. Vickerman (1988, in Thacker & Hickman 1990) suggests that reduction in abundance and activity of predatory invertebrates may lead to an increase in the frequency and magnitude of pest outbreaks. For this reason, development of less environmentally damaging pest control methods is desirable. Isman (2000) states that various plant extracts affect mortality and development of some insect pests in a similar fashion as conventional pesticides, but residues from these oils do not persist in the environment to the degree that some synthetic pesticides do. This potential for decreasing long-term effects of pest control applications makes development of plant extracts as pesticides a potentially viable option for land managers. However, in addition to testing the effects of plant extracts on insect pests, it is important to determine if the selected control candidates also negatively affect non-target organisms. For this reason, this study was designed to test the effects of grasshopper control candidates on two arthropod families that are common in this region, Carabidae (ground beetles) and Phalangidae (daddy longlegs).

Beneficial organisms play an important role in the ecosystem by breaking down plant material, feeding on dead insect remains, providing food for other

organisms, and, in the case of some carabid species, feeding on grasshopper eggs (Lövei & Sunderland 1996). Because carabids feed on invertebrate prey, including pest insects, they can potentially reduce the need for pesticide applications. Thus, the reverse may be true – negatively affecting carabid populations will reduce their feeding, thereby increasing the need for grasshopper control measures. Carabids and phalangiids were selected for this study because they have feeding behaviour that is similar to grasshoppers: they both have chewing mouthparts and feed on vegetation and/or other arthropods. Thus, these non-target organisms may be negatively affected by the grasshopper control methods used in this study. We compare the effects of plant extract applications tested as grasshopper control candidates (Chapter 2 & 3), to the effects of carbaryl bait, a carbamate pesticide used for control of grasshoppers, and an untreated control.

Materials and Methods

Selection of Control Candidates

Plant extracts used in this study were selected from oils used in field experiments in previous chapters for control of grasshoppers (Chapters 2 & 3). Additionally, preliminary laboratory tests showed that neem oil, *Azadirachta indica*, and cedarwood oil, *Cedrus deodorata*, caused grasshoppers to respond most negatively when exposed to these treatments, compared to other control candidates used in Chapter 2. Carbaryl bait (Ecobait™) was selected for this study in order to

compare the effects of a synthetic pesticide used for control of grasshoppers against the effects of the chosen plant extracts. Johnson & Henry (1987) report that carbaryl bait, when compared to spray formulations, has a reduced rate of drift and lower concentrations of active ingredients, therefore, it is a viable alternative to spray formulations of other synthetic grasshopper control methods such as cyhalothrin-lambda or deltamethrin.

Experimental Design

This experiment was conducted in August 2003 at Jefferson Community Pasture. Pitfall traps were used to collect and quantify change in numbers of carabids and phalangiids before and after treatments. Pitfall trap sampling is a commonly used method for monitoring effects of disturbances on terrestrial arthropod communities, especially ground beetles (Lövei & Sunderland 1996). Newton & Yeargan (2002) used pitfall trap sampling to examine species composition of a phalangiid species. Pitfall traps consisted of 500-ml plastic containers set into the ground so that the rim was at ground level. They were filled to ~3 cm below the rim with propylene glycol and covered with a lid that was suspended approximately 2 cm above the ground using metal rods.

Using randomised complete block design, four blocks were established with two plots of each of the three treatments and two controls in each block (8 plots per block). Each plot contained five pitfall traps in the treatment area. Plots were 100

m x 100 m (1 hectare) in size, with one pitfall trap in the centre and four satellite pitfall traps at 40 m distance from the centre trap in north, south, east and west direction (Figure 4.1). Treatment plots were separated by an untreated zone approximately 200 m wide at the closest points in order to ensure separation of treated areas. Distances were measured using handheld GPS systems mounted on all terrain vehicles (quads).

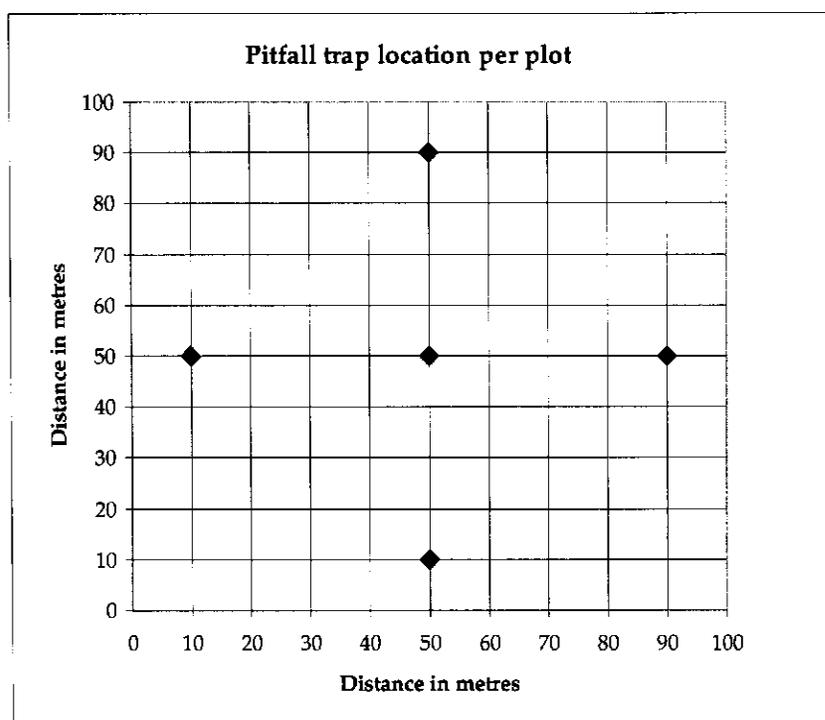


Figure 4.1. Example of pitfall trap locations in 1 hectare treatment plot

Forty pitfall traps were established in each of the four blocks with one block in each of four sections of land at Jefferson Community Pasture (8 plots x 4 blocks x 5 pitfall traps = 160 traps; N for each treatment = 40 traps). Figure 4.2 is an example of the layout of one experiment block put in the western-most section of JCP; X and Y axis are UTM coordinates (Zone 12). Two of the blocks (North and

Centre) had been hayed just before treatment and the other two (West and East) contained growth of mixed grasses: meadow brome, intermediate wheat, pubescent wheat, tall fescue and alfalfa.

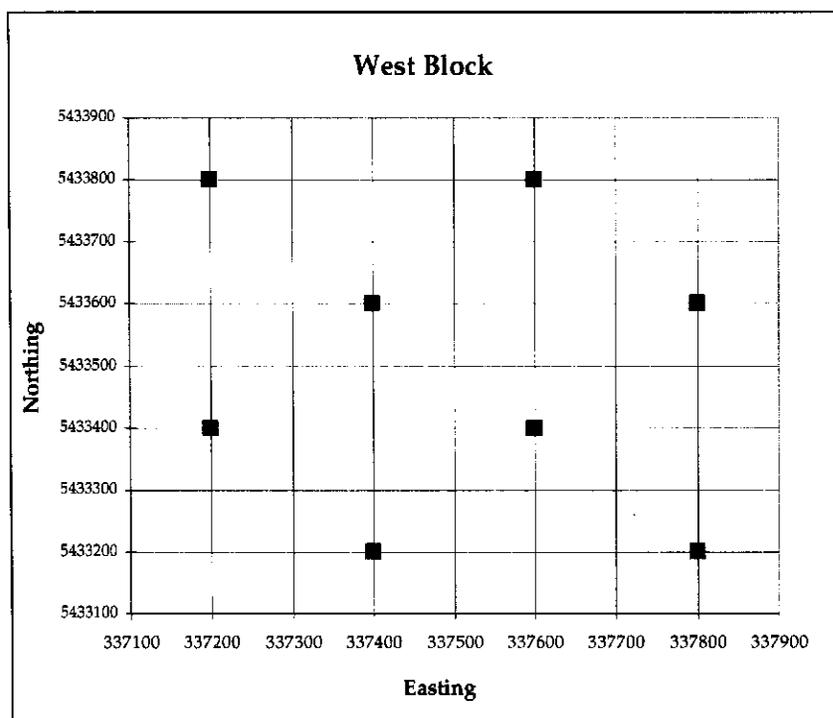


Figure 4.2. Example of one block – each square represents one hectare treatment plot (5 traps)

Formulation and Application

Carbaryl bait was applied on August 18, 2003 from 1200 h to 1800 h at a rate of 4 kg/ha (40-80g AI/ha) to all the selected plots using a truck-mounted bran spreader constructed at the Lethbridge Research Centre. Spray treatments were applied on August 19, 20 and 21, 2003, from 0600 h to 1600 h. Cedarwood oil was applied at a rate of 1.3 L/ha and neem oil was applied at a rate of 1.2 L/ha, according to applications made for grasshopper control testing in Chapter 2. Treatments were mixed with water and an emulsifier (Sunlight™ dish soap) for

total application rate of 80 L/ha. Approximately 2 ml of soap was used in each 80-litre application. Oils were emulsified in soap and water mixture by manually agitating the sprayer tank and by tank motion during application. Applications of oils were made using a Spray-Tech Systems Ltd. ATV 1810 quad-mounted sprayer with a 3 m boom. Spray jets were 50 cm from the ground. Minimal drift was observed, as winds were <15 km/h throughout the treatment period.

Ecological Sampling and Statistical Analysis

Pitfall traps were emptied approximately every five days from two weeks before treatment until two weeks after treatment. Additionally, traps were emptied directly after each of the treatment applications to ensure that untreated insects were not counted as part of the treated samples. Due to the number of pitfalls and the area covered, four field assistants assisted in the installing and emptying of the pitfall traps and two quads were used simultaneously. Collected samples were contained in one or more 100-ml alcohol vials in a freezer (-5°C) at the Lethbridge Research Centre until they were cleaned in the laboratory, separated into families, and counted. Although larval and adult carabids have similar feeding habits, only adult ground beetles were counted in this study. As phalangiids are hemimetabolous, adults and juveniles were not differentiated and all were counted. Analyses of variance and analyses covariance were conducted comparing effect of treatments against untreated controls for reduction in numbers of carabids and phalangiids after treatments.

Results

Effect – Carabidae

Table 4.1. Carabids per plot (5 traps per plot) collected from two weeks before to two weeks after treatment

| Section | Treatment | Plot | Before | After | Section | Treatment | Plot | Before | After |
|---------|-----------|------|--------|-------|---------|-----------|------|--------|-------|
| East | ecobait | 1 | 11 | 9 | North | ecobait | 1 | 7 | 5 |
| | cedar | 2 | 10 | 2 | | cedar | 2 | 6 | 7 |
| | neem | 3 | 11 | 8 | | control | 3 | 8 | 11 |
| | control | 4 | 6 | 18 | | neem | 4 | 2 | 1 |
| | cedar | 5 | 11 | 2 | | neem | 5 | 19 | 22 |
| | ecobait | 6 | 8 | 1 | | ecobait | 6 | 6 | 13 |
| | neem | 7 | 24 | 17 | | cedar | 7 | 5 | 2 |
| | control | 8 | 7 | 8 | | control | 8 | 17 | 5 |
| West | control | 1 | 9 | 17 | Centre | control | 1 | 13 | 18 |
| | neem | 2 | 11 | 4 | | neem | 2 | 38 | 39 |
| | cedar | 3 | 13 | 11 | | cedar | 3 | 22 | 6 |
| | ecobait | 4 | 20 | 18 | | ecobait | 4 | 16 | 11 |
| | cedar | 5 | 19 | 42 | | control | 5 | 8 | 9 |
| | ecobait | 6 | 9 | 1 | | neem | 6 | 59 | 19 |
| | control | 7 | 8 | 8 | | cedar | 7 | 79 | 16 |
| | neem | 8 | 6 | 5 | | ecobait | 8 | 16 | 2 |

Table 4.1 shows the absolute values for carabids before and after treatments were applied. Each plot contained 5 pitfall traps and each block consisted of two plots for each treatment (10 pitfall traps per block of each treatment). Figure 4.3 shows the mean percent reduction of carabids for each treatment with 95% confidence intervals. Although, in the absolute data (Table 4.1), numbers appear to decrease for all treatments except the control, mean percent reduction in all carabid counts per pitfall trap were negative, therefore showing an increase after treatment, with the exception of plots treated with cedarwood oil, where mean percent reduction was 1.96% (Figure 4.3). Mean percent reduction for the carbaryl bait treatment plots remained near zero (-1.96%). The control plots had the highest increase in number of carabids at -69% mean percent reduction, followed by

neem at -23%. Data were analysed using analysis of variance to compare mean percent reduction for all treatments. Effects of cedarwood oil, neem oil and carbaryl bait on carabid mortality showed no significant effect when compared to the control, $F_{(3,139)} = 1.16$, $p = 0.38$, and no significant interaction between block and treatment, $F_{(9,139)} = 1.22$, $p = 0.29$ (Table 4.2).

Table 4.2. Summary of ANOVA statistics for treatment effects on carabid abundance for all pitfall traps

| Source of variation | df | MS | F | P |
|---------------------|-----|----------|-------|-------|
| Treatment | 3 | 42692.34 | 1.162 | 0.377 |
| Block | 3 | 29324.09 | 0.798 | 0.525 |
| Block*Treatment | 9 | 36759.04 | 1.221 | 0.287 |
| Residual | 139 | 30114.60 | | |

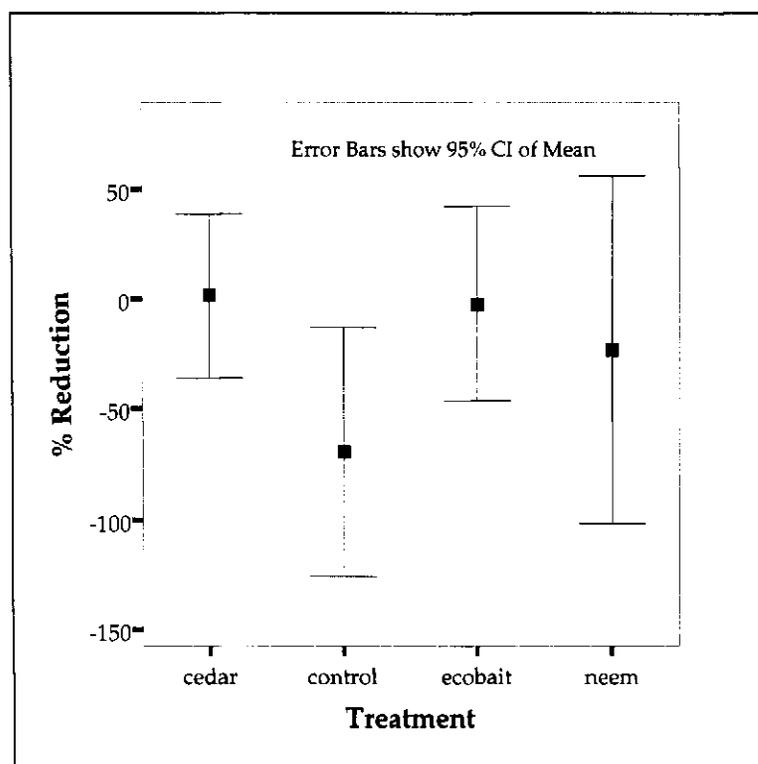


Figure 4.3. Mean percent reduction of carabids from two weeks before treatment to two weeks after treatment for each treatment with 95% confidence intervals

Data were analysed using analysis of covariance in order to account for change in carabid abundance before and after collection. Effects of cedarwood oil, neem oil and carbaryl bait on carabid mortality showed no significant effect when compared to the control, $F_{(3,138)} = 0.54$, $p = 0.67$, and a significant interaction between block and treatment, $F_{(9,138)} = 2.11$, $p = 0.03$ (Table 4.3).

Table 4.3. Summary of ANCOVA statistics for treatment effects on carabid abundance for all pitfall traps

| Source of variation | df | MS | F | P |
|---------------------------|-----|-------|-------|-------|
| Initial Count (covariate) | 1 | 84.87 | 9.319 | 0.003 |
| Treatment | 3 | 10.35 | 0.542 | 0.666 |
| Block | 3 | 9.75 | 0.522 | 0.678 |
| Block*Treatment | 9 | 19.21 | 2.109 | 0.033 |
| Residual | 138 | 9.11 | | |

As pitfall traps on the edge of the treated area were situated close to untreated terrain, these data were also analysed using only traps located in the centre of each treatment plot, excluding the north, south, east and west traps, to determine if insects in the middle of the treatment area were more affected. The results of this analysis of covariance also showed no significant main effect of treatment on carabid mortality, $F_{(3,13)} = 0.13$, $p = 0.94$, and no significant interaction between block and treatment, $F_{(9,13)} = 0.67$, $p = 0.72$ (Table 4.4).

Table 4.4. Summary of ANCOVA statistics for treatment effects on carabid abundance for centre pitfall traps only

| Source of variation | df | MS | F | P |
|---------------------------|----|-------|-------|-------|
| Initial Count (covariate) | 1 | 11.78 | 0.718 | 0.412 |
| Treatment | 3 | 1.51 | 0.133 | 0.938 |
| Block | 3 | 7.75 | 0.688 | 0.579 |
| Block*Treatment | 9 | 11.04 | 0.673 | 0.720 |
| Residual | 13 | 16.40 | | |

In order to account for change in the control plots in relation to the treatment plots, Abbott's adjusted percent mortality was used. This adjustment uses a cross-product calculation $((T2 \cdot C1) / (T1 \cdot C2))$ where T = treatment; C = control and 1 = before; 2 = after) in order accurately compare change in insect abundance before and after treatments in relation to change in insect abundance in the control plots (Abbott 1925). These data are displayed in Table 4.5 showing average numbers of carabids before and after treatments in each block. The overall averages for the four blocks combined are shown in Table 4.6. With the exception of the cedarwood treatment in the west block (W) and all treatments in the north block (N), carabid abundance shows an overall reduction, after adjusting for change in control. This is evidenced further in Table 4.6 where percent reduction in carabid abundance varies from ~45 to 57% for all three treatments.

Table 4.5. Abbott's adjusted percent mortality for carabids shown for each block

| N | | | | | C | | | | |
|---------|--------|-------|-------------------------|-----------|---------|--------|-------|-------------------------|-----------|
| SUMS | before | after | adjusted % reduction | Std Error | SUMS | before | after | adjusted % reduction | Std Error |
| ecobait | 13 | 18 | -116.35 | 104.87 | ecobait | 32 | 13 | 68.40 | 13.88 |
| cedar | 11 | 9 | -27.84 | 70.55 | cedar | 101 | 22 | 83.06 | 6.34 |
| neem | 21 | 23 | -71.13 | 75.30 | neem | 97 | 58 | 53.49 | 15.58 |
| control | 25 | 16 | | | control | 21 | 27 | | |

| E | | | | | W | | | | |
|---------|--------|-------|-------------------------|-----------|---------|--------|-------|-------------------------|-----------|
| SUMS | before | after | adjusted % reduction | Std Error | SUMS | before | after | adjusted % reduction | Std Error |
| ecobait | 19 | 10 | 73.68 | 13.62 | ecobait | 29 | 19 | 55.45 | 19.21 |
| cedar | 21 | 4 | 90.48 | 6.12 | cedar | 32 | 53 | -12.63 | 43.47 |
| neem | 35 | 25 | 64.29 | 15.32 | neem | 17 | 9 | 64.00 | 18.66 |
| control | 13 | 26 | | | control | 17 | 25 | | |

Table 4.6. Abbott's adjusted percent mortality for carabids shown overall (blocks combined)

| | SUMS | before | after | adjusted % reduction | Std Error |
|---------|------|--------|-------|-------------------------|-----------|
| ecobait | 93 | 60 | 47.84 | 11.80 | |
| cedar | 165 | 88 | 56.88 | 8.75 | |
| neem | 170 | 115 | 45.31 | 10.71 | |
| control | 76 | 94 | | | |

Effect – Phalangiidae

Table 4.7. Phalangiids per plot (5 traps per plot) collected from two weeks before to two weeks after treatment

| Section | Treatment | Plot | Before | After | Section | Treatment | Plot | Before | After |
|---------|-----------|------|--------|-------|---------|-----------|------|--------|-------|
| East | ecobait | 1 | 17 | 18 | North | ecobait | 1 | 79 | 90 |
| | cedar | 2 | 83 | 32 | | cedar | 2 | 118 | 65 |
| | neem | 3 | 111 | 46 | | control | 3 | 154 | 137 |
| | control | 4 | 37 | 57 | | neem | 4 | 116 | 57 |
| | cedar | 5 | 45 | 56 | | neem | 5 | 57 | 29 |
| | ecobait | 6 | 54 | 7 | | ecobait | 6 | 85 | 122 |
| | neem | 7 | 127 | 58 | | cedar | 7 | 32 | 104 |
| | control | 8 | 33 | 30 | | control | 8 | 141 | 107 |
| West | control | 1 | 71 | 55 | Centre | control | 1 | 142 | 70 |
| | neem | 2 | 66 | 28 | | neem | 2 | 269 | 162 |
| | cedar | 3 | 48 | 33 | | cedar | 3 | 130 | 30 |
| | ecobait | 4 | 29 | 23 | | ecobait | 4 | 26 | 10 |
| | cedar | 5 | 57 | 49 | | control | 5 | 45 | 36 |
| | ecobait | 6 | 16 | 48 | | neem | 6 | 171 | 58 |
| | control | 7 | 49 | 99 | | cedar | 7 | 302 | 92 |
| | neem | 8 | 26 | 53 | | ecobait | 8 | 155 | 46 |

Table 4.7 shows the absolute values for phalangiids before and after treatments were applied. Each plot contained 5 pitfall traps and each block consisted of two plots for each treatment (10 pitfall traps per block of each treatment). Figure 4.4 shows the mean percent reduction of phalangiids for each treatment with 95% confidence intervals. In all treatments and controls, mean number of phalangiids increased after treatment with the exception of neem, which decreased by 21%. Phalangiid numbers showed the highest increase overall in the cedar plots at 86%, followed by Ecobait™ at 54%, and control plots at 49%. In conformity with the statistical results for the carabid experiment, effects of cedarwood oil, neem oil and carbaryl bait on phalangiid mortality showed no significant effect when compared to control, $F_{(3,141)} = 0.66$, $p = 0.60$, and no significant interaction between block and treatment, $F_{(9,141)} = 1.89$, $p = 0.06$ (Table 4.8).

Table 4.8. Summary of ANOVA statistics for treatment effects on phalangiid abundance for all pitfall traps

| Source of variation | df | MS | F | P |
|---------------------|-----|-----------|-------|-------|
| Treatment | 3 | 78041.15 | 0.657 | 0.599 |
| Block | 3 | 204232.84 | 1.719 | 0.232 |
| Block*Treatment | 9 | 118831.85 | 1.890 | 0.058 |
| Residual | 141 | 62862.91 | | |

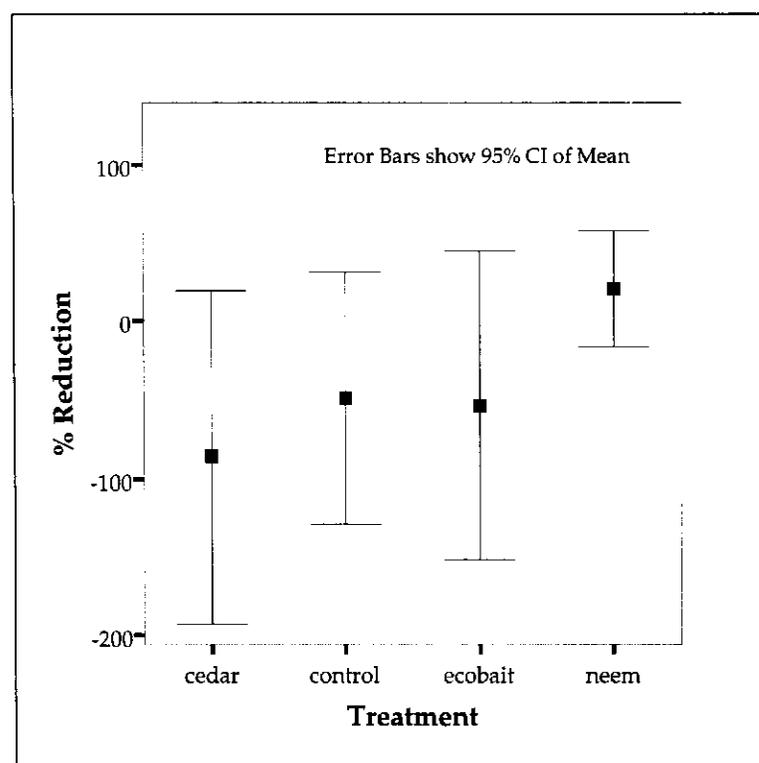


Figure 4.4. Mean percent reduction of phalangiids from two weeks before treatment to two weeks after treatment for each treatment with 95% confidence intervals

Data were analysed using analysis of covariance in order to account for change in carabid abundance before and after collection. Effects of cedarwood oil, neem oil and carbaryl bait on phalangiid mortality showed no significant effect when compared to the control, $F_{(3,140)} = 1.37$, $p = 0.31$, and a significant interaction between block and treatment, $F_{(9,140)} = 2.08$, $p = 0.04$ (Table 4.9).

Table 4.9. Summary of ANCOVA statistics for treatment effects on phalangiid abundance for all pitfall traps

| Source of variation | df | MS | F | P |
|---------------------------|-----|---------|-------|-------|
| Initial Count (covariate) | 1 | 2864.11 | 32.93 | 0.000 |
| Treatment | 3 | 246.51 | 1.37 | 0.312 |
| Block | 3 | 607.26 | 3.51 | 0.058 |
| Block*Treatment | 9 | 180.93 | 2.08 | 0.035 |
| Residual | 140 | 86.96 | | |

As pitfall traps on the edge of the treated area were situated close to untreated terrain, these data were analysed using only traps located in the centre of each treatment plot, excluding the north, south, east and west traps, to determine if insects in the middle of the treatment area were more affected. The results of this analysis of covariance also showed no significant main effect of treatment on phalangiid mortality, $F_{(3,14)} = 0.64$, $p = 0.61$, and no significant interaction between block and treatment, $F_{(9,14)} = 1.39$, $p = 0.28$ (Table 4.10).

Table 4.10. Summary of ANCOVA statistics for treatment effects on phalangiid abundance for centre pitfall traps only

| Source of variation | df | MS | F | P |
|---------------------------|----|--------|-------|-------|
| Initial Count (covariate) | 1 | 495.95 | 2.792 | 0.117 |
| Treatment | 3 | 155.01 | 0.642 | 0.605 |
| Block | 3 | 253.42 | 1.062 | 0.405 |
| Block*Treatment | 9 | 246.99 | 1.391 | 0.280 |
| Residual | 14 | 177.61 | | |

In order to account for change in the control plots in relation to the treatment plots, Abbott's adjusted percent mortality was applied to phalangiid data. These data are displayed in Table 4.11 showing average numbers of phalangiids before and after treatments in each block. The overall averages for the four blocks

combined are shown in Table 4.12. Adjusted percent mortality varies among blocks where Ecobait™ showed an increase in phalangiid abundance in the west and north blocks (W and N), but a decrease in phalangiid abundance in the east and centre blocks (E and C) (Table 4.11). There was also some variation among blocks with regard to the cedarwood treatments showing that abundance decreased in all blocks except the north, where abundance increased by 36%. However, Table 4.12 shows a decrease in abundance for all treatments (10-41%) when blocks are combined and adjusted percent mortality is calculated overall.

Table 4.11. Abbott's adjusted percent mortality for phalangiids shown for each block

| N | | | | | C | | | | |
|---------|--------|-------|----------------------|-----------|---------|--------|-------|----------------------|-----------|
| SUMS | before | after | adjusted % reduction | Std Error | SUMS | before | after | adjusted % reduction | Std Error |
| ecobait | 164 | 212 | -56.29 | 21.14 | ecobait | 181 | 56 | 45.42 | 10.66 |
| cedar | 150 | 169 | -36.22 | 19.30 | cedar | 432 | 122 | 50.18 | 7.92 |
| neem | 173 | 86 | 39.90 | 9.48 | neem | 440 | 220 | 11.79 | 12.96 |
| control | 295 | 244 | | | control | 187 | 106 | | |

| E | | | | | W | | | | |
|---------|--------|-------|----------------------|-----------|---------|--------|-------|----------------------|-----------|
| SUMS | before | after | adjusted % reduction | Std Error | SUMS | before | after | adjusted % reduction | Std Error |
| ecobait | 71 | 25 | 71.67 | 8.01 | ecobait | 45 | 71 | -22.94 | 23.66 |
| cedar | 128 | 88 | 44.68 | 11.73 | cedar | 105 | 82 | 39.15 | 11.63 |
| neem | 238 | 104 | 64.84 | 7.00 | neem | 92 | 81 | 31.39 | 13.38 |
| control | 70 | 87 | | | control | 120 | 154 | | |

Table 4.12. Abbott's adjusted percent mortality for phalangiids shown overall (blocks combined)

| SUMS | before | after | adjusted % reduction | Std Error |
|---------|--------|-------|----------------------|-----------|
| ecobait | 461 | 364 | 10.22 | 8.08 |
| cedar | 815 | 461 | 35.68 | 5.22 |
| neem | 943 | 491 | 40.80 | 4.69 |
| control | 672 | 591 | | |

Discussion

Based on the results of the ANCOVA tests, there is no significant effect of cedarwood oil, neem oil, or carbaryl bait on carabid or phalangiid mortality. Overall percent reduction per pitfall trap indicated that both carabid and phalangiid numbers increased after treatment, with the exception of carabids treated with cedarwood oil (2% reduction) and phalangiids treated with neem oil (21% reduction). The mean percent reduction for control plots in the carabid samples showed a larger increase than mean percent reductions for all treated plots (Figure 4.3). These data are highly variable showing large confidence intervals and, therefore, little statistical difference between means for each treatment. The significance between block and treatment interaction found in the analysis of covariance (Table 4.3) is explained in Table 4.4, where individual block results show that the north block behaved differently from the other three blocks. The adjusted percent mortality shown in Tables 4.5 and 4.6 indicate a 45-57% decrease in carabid abundance for all treatments. While not a statistical test, Abbott's adjusted percent mortality has been shown to indicate the relative degree of reduction in simulation studies (Schaalje *et al.* 1986). These results suggest that accounting for change in control in relation to change in treatment plots is important in determining effect of treatment. Quinn *et al.* (1991) used carbaryl-bran bait (1.5 kg/ha) in a non-target study of carabids and found that the bait treatments significantly affected carabid communities, however, these effects were not evident one year after treatment. Survey of current literature

found no studies describing effects of field tests of neem and cedarwood oil on carabid abundance.

The mean number of phalangiids increased more for plots treated with cedarwood and Ecobait™ than those of the control (Figure 4.4). These data are highly variable showing large confidence intervals and, therefore, little statistical difference between mean percent reductions for each treatment. Although the mean number of phalangiids treated with neem oil decreased by 21%, the overall increase in phalangiids and the variability in these data reflect that, when compared to the mean for the control plots, the selected control candidates do not significantly reduce phalangiid abundance. The significant block and treatment interaction found in the analysis of covariance (Table 4.9) is explained in Table 4.11, where individual block results show that the north block and west block behaved differently from the other blocks. However, after adjusting for change in control relative to change in treatment plots, treatment plots show a 10-41% decrease in phalangiid abundance (Table 4.12). Again, these results suggest the need for accounting for relative change in control to determine if, indeed, an effect of treatments has occurred. Additionally, Thacker & Hickman (1990) recommend that sublethal behavioural effects of treatments should also be studied to determine if there is an effect of decreased predation efficiency, and, therefore, decreased reproductive fitness in non-target organisms. This is beyond the scope of this study yet points to the potential for negative effects and the

possibility that even if direct mortality is not significantly affected by the substances tested here, a long term study may show a different result.

Conclusions

The aim of this study was to determine if the selected grasshopper control methods used at the Jefferson Community Pasture could significantly affect mortality of arthropods beneficial to the ecological integrity of the site. Although the results of the ANCOVA indicate that these treatments, applied at the above rates, do not negatively affect carabids or phalangiids, the adjusted percent mortality suggests otherwise. Further development of control candidates used in this study for grasshopper control will require continued evaluation of the effects of these treatments on non-target organisms. Development of new pesticides involves not only discovering effects of treatments on the targeted organisms, but also investigating other environmental effects that could have negative impacts on the ecosystem as a whole. Hence, testing of new pesticides should be a systematic process; first selecting control candidates and determining their initial effects on the target species, and then continuing to modify formulations and concentrations in order to develop controls that are effective to the targeted pest without posing short-or long-term threats to non-target organisms.

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Chapter 5: Using Geographic Information Systems to estimate grasshopper spatial distribution and infestation levels at Jefferson Community Pasture

Abstract

Geographic Information Systems (GIS) have been used to estimate grasshopper abundance over the past two decades in order to predict the threat of pest outbreaks to landowners and to enable managers to target pesticide usage to areas that have high infestation levels. Using GIS as a management tool can benefit landowners and the environment by reducing unnecessary spraying in areas that do not contain high numbers of pests, and by preventing small concentrations from dispersing to infest large areas. By targeting areas that are infested with grasshoppers, land managers can reduce pesticide use and application cost, which reduces the amount of pesticide residues in the environment and decreases the threat to beneficial organisms. At Jefferson Community Pasture (JCP) grasshopper pests caused considerable damage on field edges in the summer of 2002. The purpose of this study was to create grasshopper density maps for the summers of 2003 and 2004 to show areas where grasshoppers were particularly abundant so that Ducks Unlimited Canada property managers can target their grasshopper control measures accordingly.

Introduction

In hot, dry years, grasshopper outbreaks have significant economic implications for landowners on the southern prairies. Environmental factors such as heat accumulation, rainfall, soil quality and vegetation can affect the severity of grasshopper infestations, as well as insect reproduction rate (Johnson *et al.* 1996). By targeting areas that are infested with grasshoppers, land managers can reduce pesticide use and application cost, which reduces the amount of pesticide residues in the environment and decreases the threat to beneficial organisms. Geographic Information Systems (GIS) have been used to estimate grasshopper abundance over the past two decades in order to predict the threat of pest outbreaks to landowners and to enable managers to target pesticide use to areas that have high infestation levels. When evaluating GIS models used to predict grasshopper outbreaks, Johnson (1989) found a strong correlation between predicted and observed maps. Hence, collecting grasshopper abundance data and inputting counts into a GIS in order to estimate potential threat of grasshopper infestations can allow landowners to predict high infestation areas and target spraying accordingly. Using GIS as a management tool can benefit landowners and the environment by reducing unnecessary spraying in areas that do not contain large numbers of pest species.

At Jefferson Community Pasture (JCP: Figure 1.2, Chapter 1) grasshopper pests caused considerable damage on field edges in the summer of 2002 (Johnson 2004,

pers. comm.). The purpose of this study was to create grasshopper density maps for the summers of 2003 and 2004 to show areas where grasshoppers were particularly abundant so that Ducks Unlimited Canada property managers can target their grasshopper control measures accordingly.

Materials and Methods

Data were collected at JCP in the summer of 2003 and 2004 using handheld Garmin eTrex® Global Positioning Systems (GPS) to get location coordinates for each point count. Chapter 1 (Figures 1.1 & 1.2) show the location and layout of JCP. Abundance was measured by counting grasshoppers in a m² area, or, when grasshoppers were particularly abundant, measuring a quarter metre area and then multiplying by four. Data collection in 2003 was conducted predominantly by collecting roadside and field margin counts around the perimeter of JCP with few point counts made in the centre of each of the sections contained in the DUC property. These data were collected on July 17, 2003. In 2004, grasshopper density data were collected on July 22, 28 and August 11, 29. These data are comprised of roadside counts and field counts in approximately regular 4-point grids with point spacing of about 400 m, and a fifth centre at ~280 m from the corner points as depicted in Figure 5.1. These grids represent each of the 4 JCP sections (Figure 1.2, Chapter 1), as well as a half section in the far east of JCP. Additional to these regular grids, several roadside counts were made, therefore,

on each day that density data were collected in 2004, between 22 and 35 point counts were made.

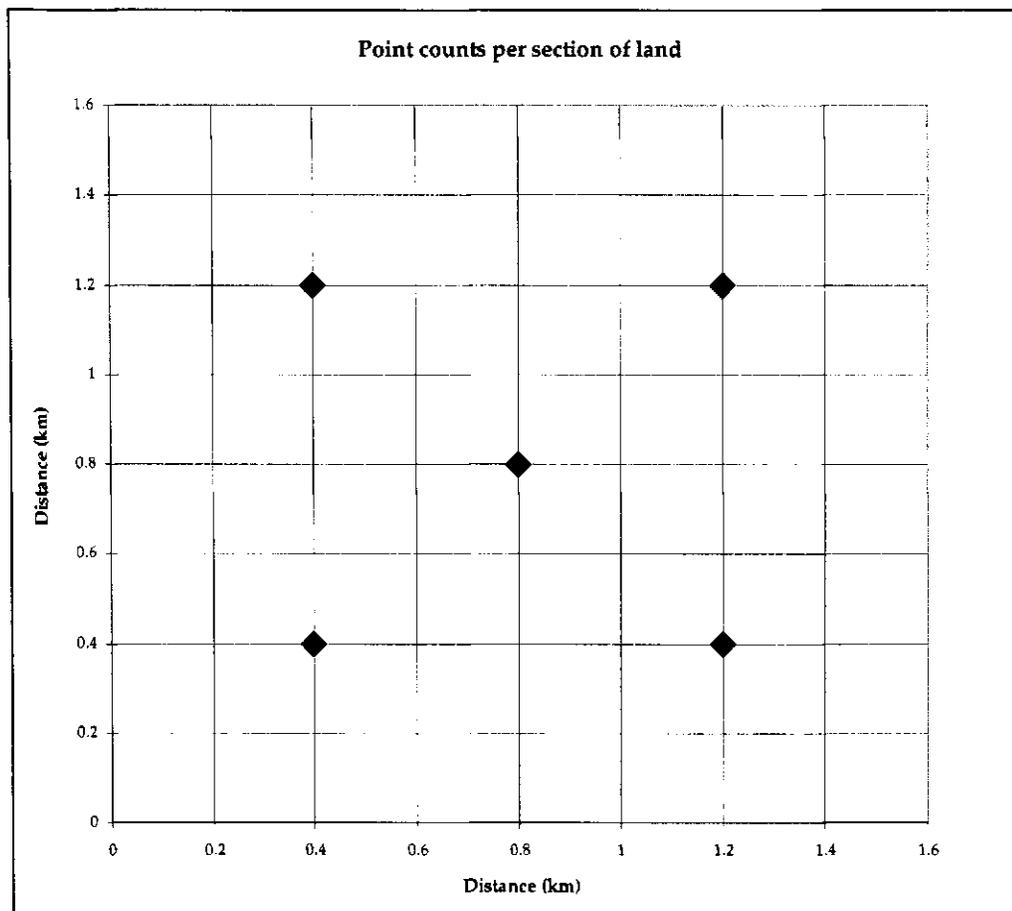


Figure 5.1. Grasshopper density point counts grid for 2004.

Data were input into a GIS and then interpolated to make five contour maps (one for each day of observations) using ArcGIS version 9 software. Ordinary kriging was used to interpolate the map surface and then values for levels of infestation were assigned to the number of grasshoppers per m² based on adult density levels cited in Johnson *et al.* (1996): 0-4 Very Light, 4-8 Light; 8-12 Moderate; 12-24 Severe; >24 Very Severe.

Results

The following maps show the results of using ordinary kriging to predict the grasshopper abundance at JCP in 2003 and 2004. Figures 5.2 to 5.6 are the interpolated surfaces of grasshopper point counts collected at JCP. Kriging was done without taking into account the presence of lakes, and the lakes on these maps are overlain onto the continuous surface of the kriged classification.

Figure 5.2 is the interpolated surface showing grasshopper infestation levels at JCP on July 17, 2003. This map surface is largely classified as "Very Severe", with some "Severe" sections, indicating high numbers of grasshoppers throughout the study area (12-24+ per m²). The sample points were overlain on this map because the area was not sampled using the grid format shown in Figure 5.1. The interpolated surface showing grasshopper densities is not as smooth as the other interpolated surface because data collection was not as systematic.

Figure 5.3 is the interpolated surface showing grasshopper infestation levels at JCP on July 22, 2004. This map surface is of the classes "Light" and "Moderate" on the bottom portion of JCP (4-12 grasshoppers per m²). The areas in the north section indicate classifications of "Severe" and "Very Severe" infestation levels (12-24+ grasshoppers per m²).

Figure 5.4 is the interpolated surface showing grasshopper infestation levels at JCP on July 28, 2004. This map surface is largely made up of "Light" and "Moderate" levels of infestation on the southern portion of JCP. The west side in

the north section indicates a classification of “Severe” infestation level of grasshoppers (12-24 per m²).

Figure 5.5 is the interpolated surface showing grasshopper infestation levels at JCP on August 11, 2004. This map surface indicates a “Moderate” and “Severe” infestation of grasshoppers (8-24 per m²). The area in the southwest corner of the north section indicates a “Severe” infestation of grasshoppers (>24 per m²).

Figure 5.6 is the interpolated surface showing grasshopper infestation levels at JCP on August 29, 2004. This map surface indicates classes of “Moderate” and “Severe” infestation levels throughout most of the study area (8-24 grasshoppers per m²). The south-central section of JCP is classified as “Light”, indicating a lower infestation level in this area (4-8 grasshoppers per m²).

Discussion

The results of the maps produced from data collected at JCP in the summer of 2003 and 2004 show that grasshopper infestations were greater in July 2003 than in 2004. The red surface interpolated from data collected on July 17, 2003 (Figure 5.2) indicates that grasshopper densities were predicted to be greater than 24 grasshoppers per m² on the majority of the DUC property. Heat accumulation positively and rainfall negatively affect the severity of grasshopper infestations (Johnson *et al.* 1996) and the summer of 2004 had higher amounts of rainfall than that of 2003 (Figures 5.7a & b).

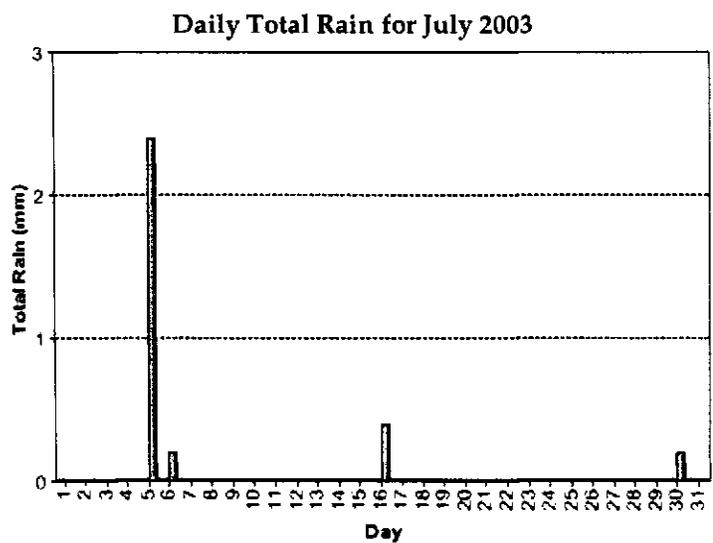


Figure 5.7a. Rainfall in Cardston, AB for July 2003

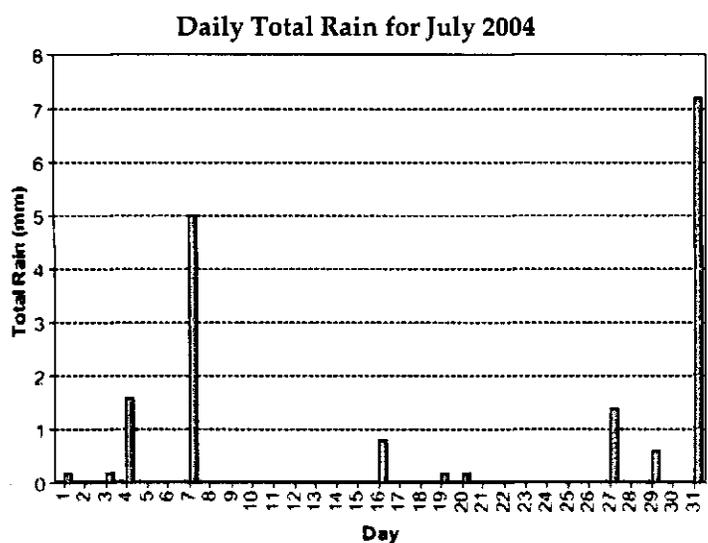


Figure 5.7b. Rainfall in Cardston, AB for July 2004

Daily precipitation data and graphs from Environment Canada (2005).
 Note the difference in y-axes scale.

Figure 5.7a and 5.7b show the daily rainfall for July 2003 and 2004. As seen in the rainfall graphs, there was more rainfall in July of 2004 than in July 2003. More moisture in 2004 may explain the results of the interpolation maps for July 22

and 28 (Figures 5.3 and 5.4) showing grasshopper infestations that were Light and Moderate for most of JCP.

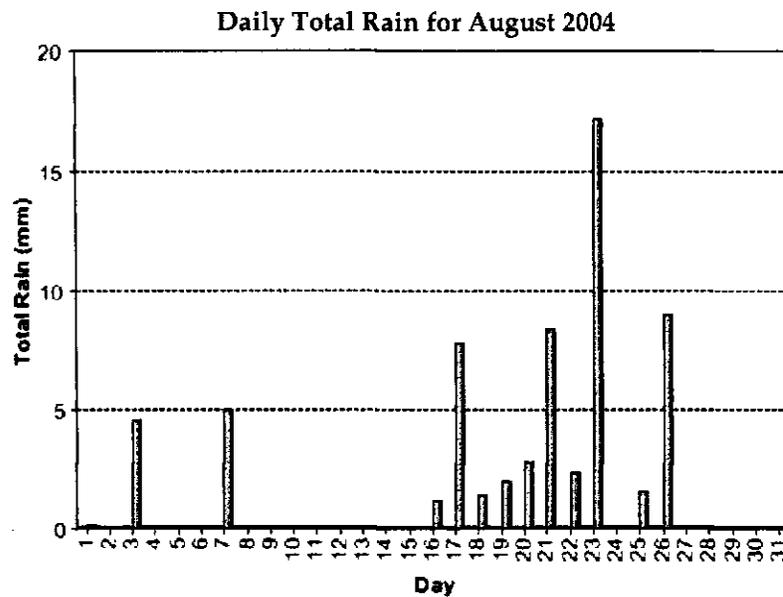


Figure 5.8. Rainfall in Cardston, AB for August 2004
Daily precipitation data and graphs from Environment Canada (2005)

According to the interpolated surfaces for 2004, August 11 and 29 (Figures 5.5 and 5.6) had elevated grasshopper densities as compared to infestation levels in July, with a slight decrease in densities from August 11 to August 29. Figure 5.8 shows an extended period of rainfall in August from the 16th to the 27th. Johnson (1988) states that changes in abundance of adult grasshoppers relate to rainfall during the period of development; populations tend to decline in areas of above-average rainfall. Rainfall in the summer of 2004 was frequent and fluctuation of grasshopper abundance may have been a result of this.

When comparing the five interpolated surfaces, data collection methods must be considered. The jagged surface seen on the map for July 17, 2003 results from uneven data collection: most data were collected from the edges of JCP, with few sample points taken from the centre of each section of land. This is indicated by the data collection points overlain in Figure 5.2. Collection of data points that covered the expanse of JCP would make for a more accurate estimate of grasshopper densities throughout the property. Grasshopper abundance data collected for the summer of 2004 provided a better coverage of each section of land included in JCP and, therefore, the maps representing grasshopper infestation levels in 2004 show a smoother surface. The kriging interpolation process uses between two and five neighbouring points, therefore, where there are fewer points a surface may be under- or overestimated depending on the surrounding points. Johnson (1993) states that high roadside counts can overestimate field counts. While this may be the case for the interpolated surface for July 17, 2003, grasshoppers abundance was indeed greater in the summer of 2003 than that of 2004 and Figures 5.2 through 5.6 illustrate that fact.

In comparing grasshopper infestation levels at JCP for the summer of 2004, there appears to be no distinct spatio-temporal pattern when examining only abundance. The predicted surface for July 22 (Figure 5.3) show higher infestation levels than for July 28 (Figure 5.4). August 11 (Figure 5.5) shows higher infestation levels than both of the July maps, and higher infestation levels than

that of August 29 (Figure 5.6). This lack of correlation between maps of grasshopper abundance may be explained by rainfall and heat accumulation. By taking these environmental factors into consideration, grasshopper infestation levels may be predicted more accurately. The spatial pattern that is suggested by these four interpolated surfaces is that grasshopper abundance tends to be greater in the northern-most section of JCP and tends to be lower in the south part of the centre section of JCP.

Conclusions

For land managers such as Ducks Unlimited Canada, systematic data collection of grasshopper abundance input into a GIS could allow for targeting of control measures that would enable managers to focus control efforts on areas of high densities while leaving areas that pose no threat to crop damage untreated. Consideration and incorporation of weather data could assist in giving an accurate prediction of times when controls may be needed and areas that may be hotspots containing higher infestation levels. Application of GIS could allow land managers to streamline grasshopper control measures, thereby reducing pesticide use and thus reducing overall cost.

JCP Grasshopper Infestation Level 17 July 2003

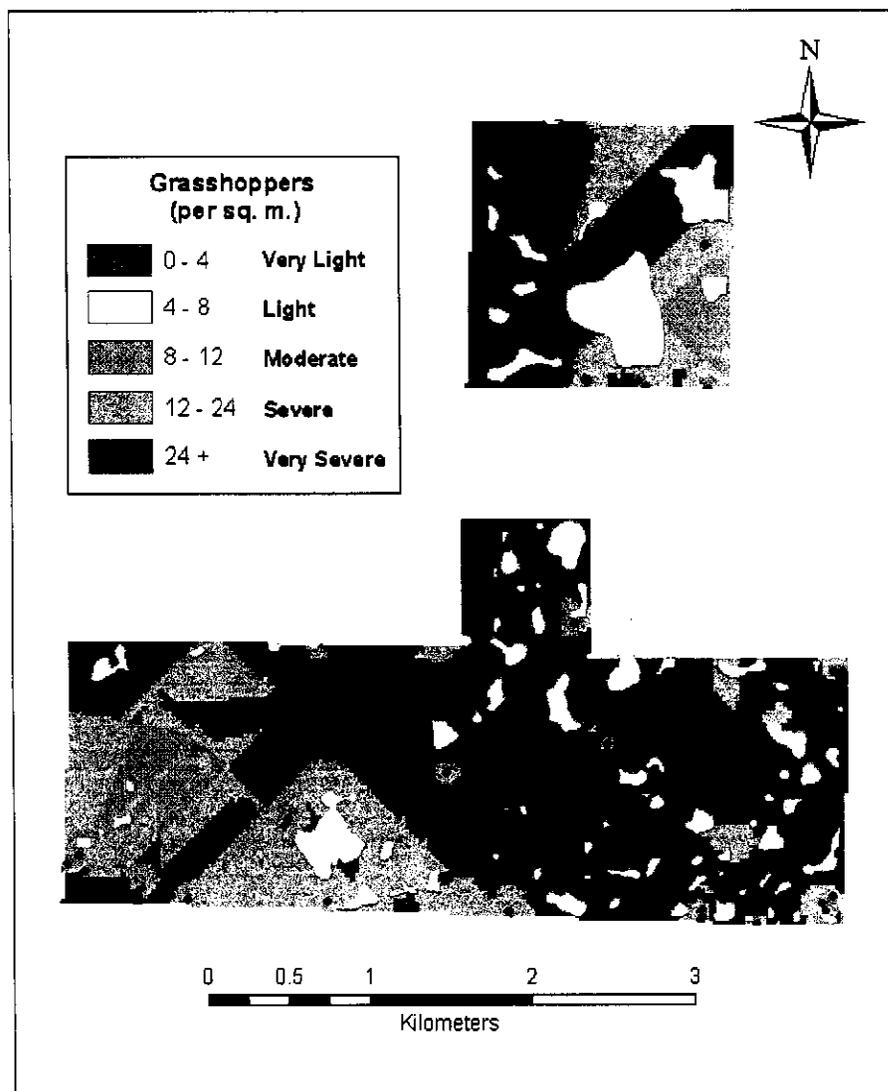


Figure 5.2. Kriged GIS map of grasshopper abundance in the study area; sampled points & lakes are overlain

JCP Grasshopper Infestation Level 22 July 04

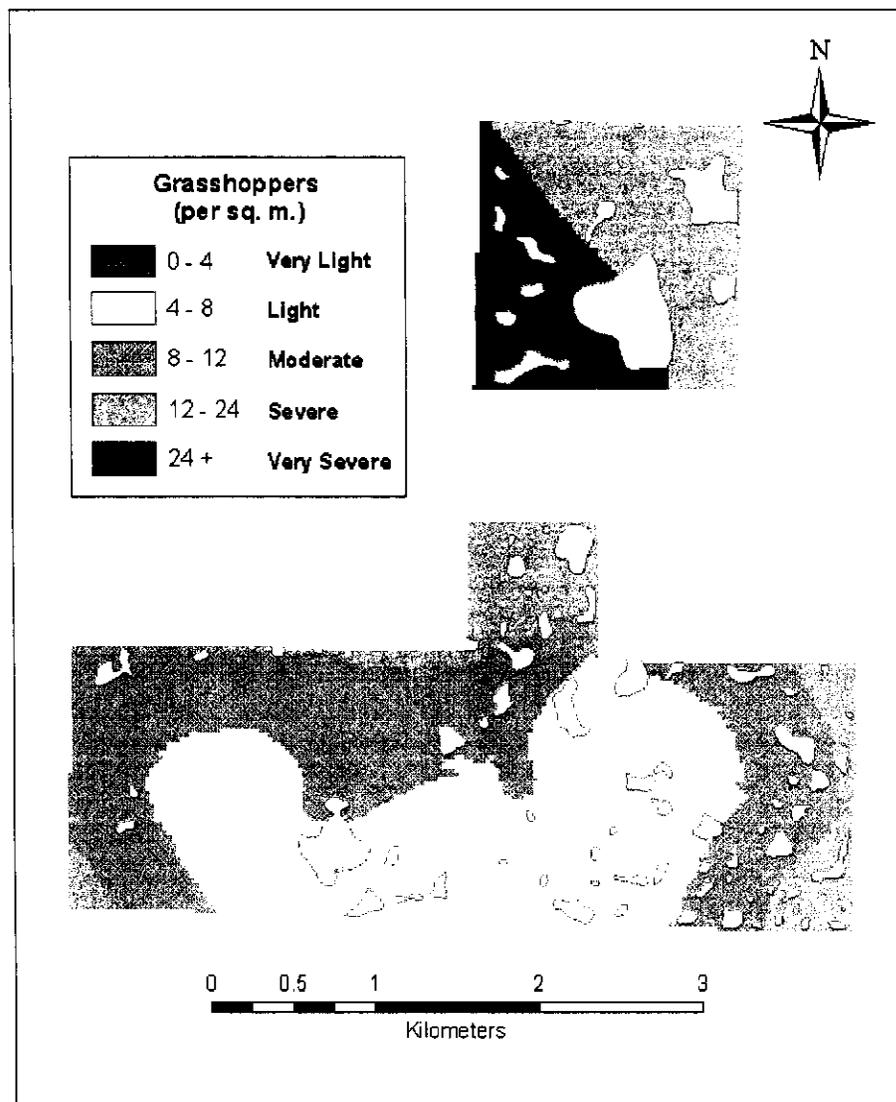


Figure 5.3. Kriged GIS map of grasshopper abundance in the study area; lakes are overlain

JCP Grasshopper Infestation Level 28 July 04

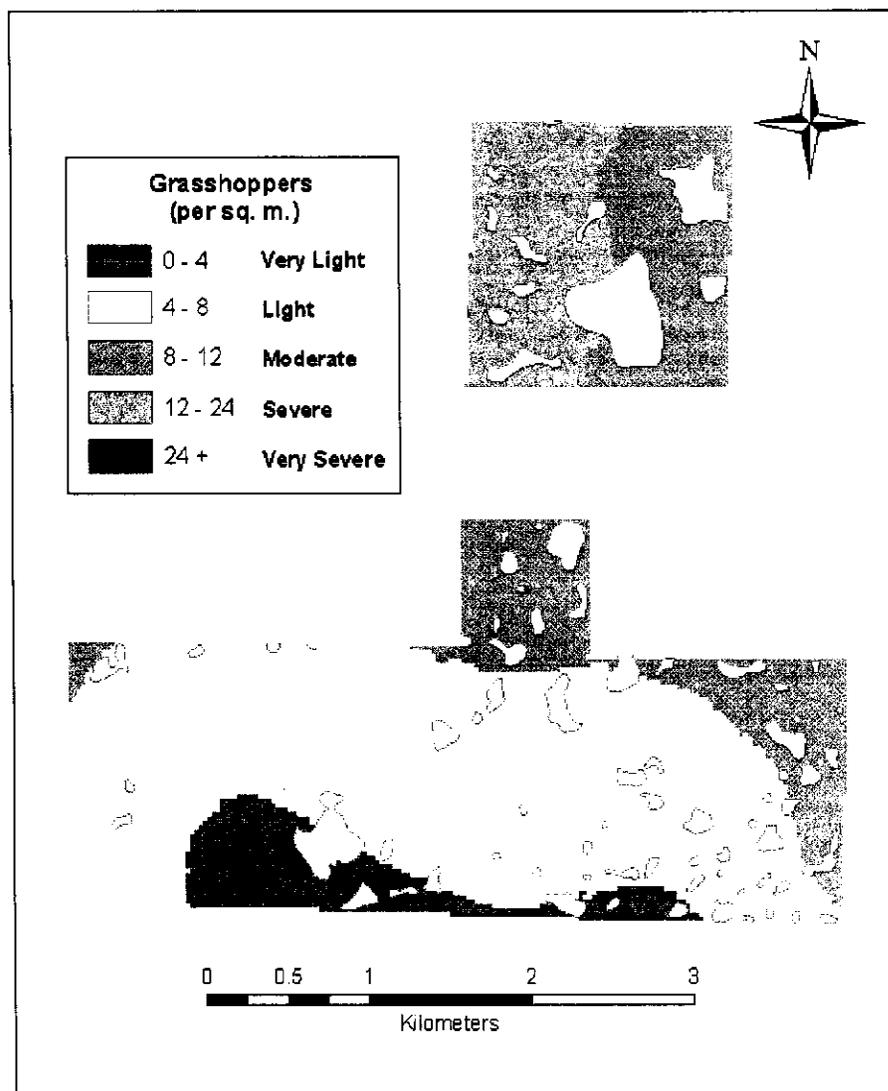


Figure 5.4. Kriged GIS map of grasshopper abundance in the study area; lakes are overlain

JCP Grasshopper Infestation Level 11 August 04

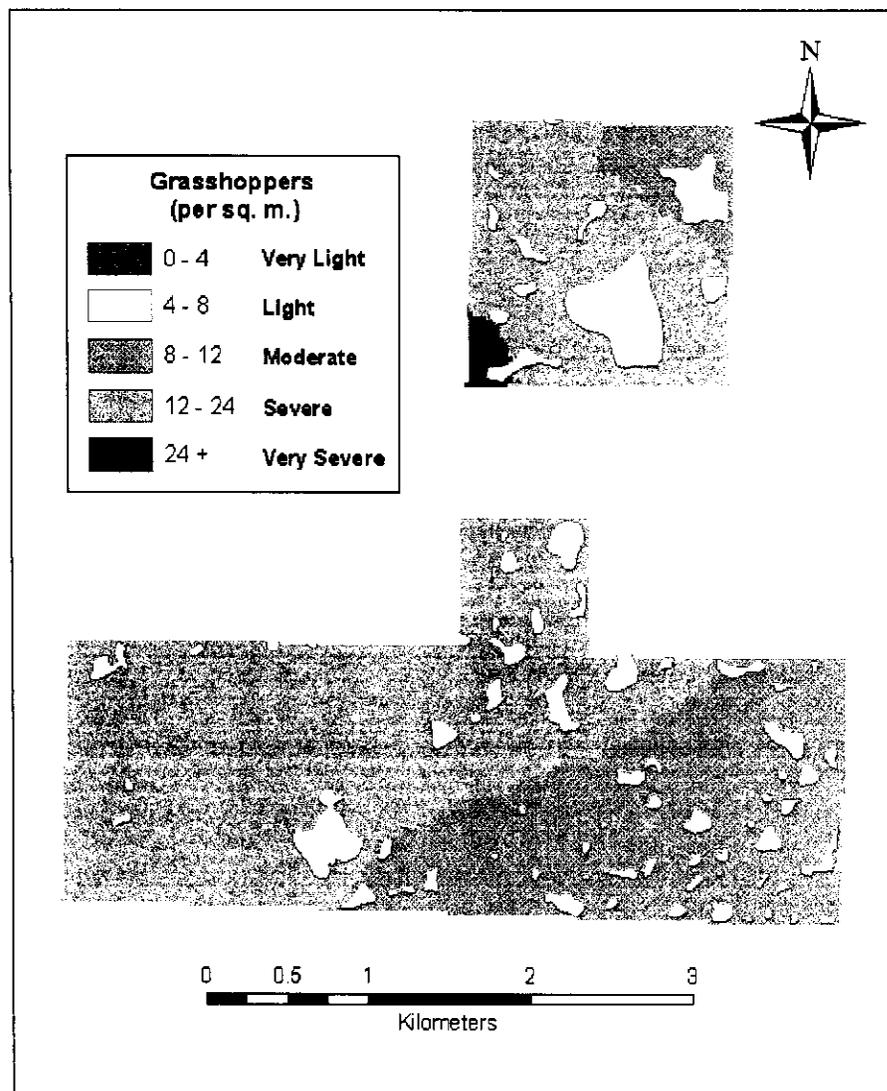


Figure 5.5. Kriged GIS map of grasshopper abundance in the study area; lakes are overlain

JCP Grasshopper Infestation Level 29 August 04

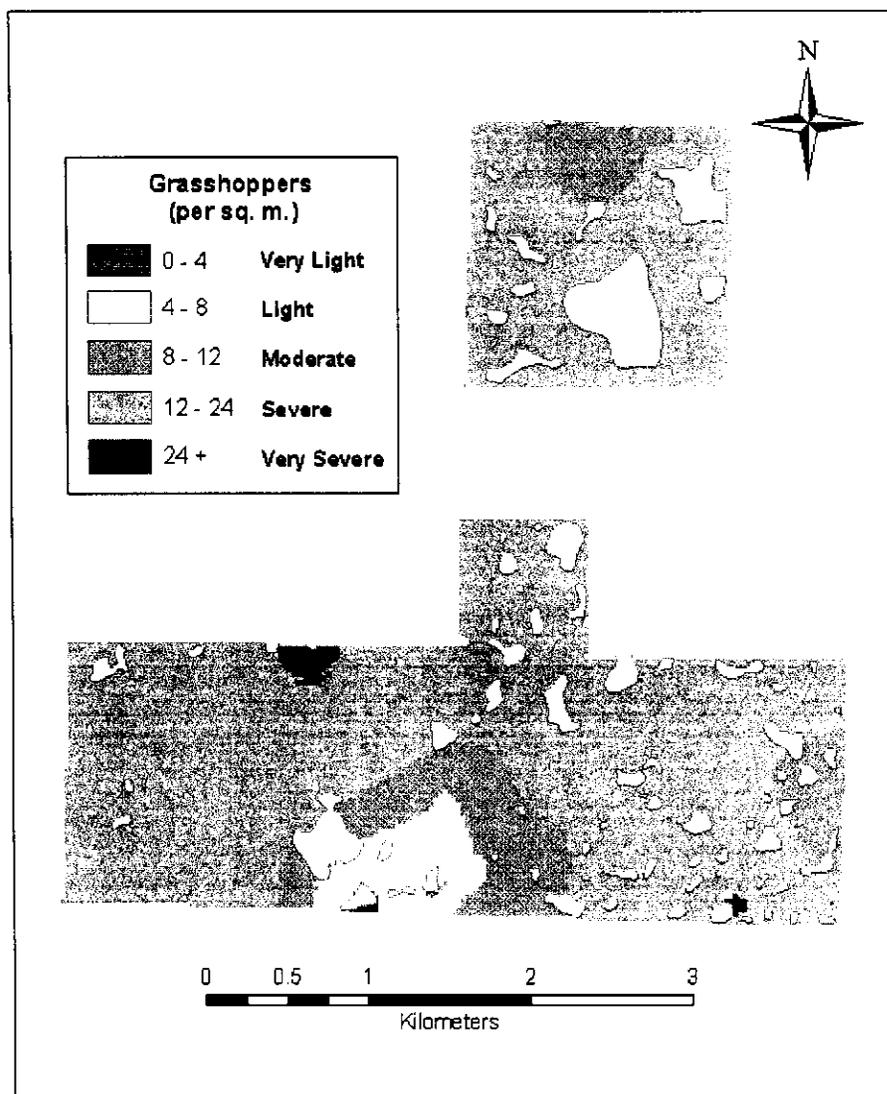


Figure 5.6. Kriged GIS map of grasshopper abundance in the study area; lakes are overlain

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Chapter 6: Summary and Conclusions

Summary

The principle objective of this thesis was to propose and test potential alternatives to synthetic grasshopper control methods. The results of the four experiments conducted in this research assessed some effects of the selected alternative control methods applied to grasshoppers and two non-target arthropods in southern Alberta. Additionally, a Geographic Information System (GIS) was used to illustrate and investigate temporal change of spatial patterns in grasshopper abundance at Jefferson Community Pasture (JCP), southern Alberta, Canada, and to explore GIS as a possible grasshopper management tool.

The experiments testing the effects of plant extracts on grasshoppers included two field studies at the JCP (Chapter 2: Long-plot Study and Field Study) and one greenhouse study conducted at the Lethbridge Research Centre (Chapter 3: Cage Study). All three of these experiments found no significant effect of any of the treatment candidates on grasshopper mortality at the operational field scale. Lab tests conducted prior to testing the selected plant extracts in the field indicated that cedarwood, rosemary and neem oil extracts showed the most potential for negatively affecting grasshopper survival. Although results from the two field experiments and the greenhouse experiment were not statistically significant, increase in grasshopper abundance was lowest in plots treated with neem (92%), cedarwood (96%) and rosemary oil (121%) in the long-plot study. In

the field study, tea tree and neem oil had the largest effect on grasshopper mortality; 62% and 56% reduction in mean number of grasshoppers per m², respectively. The cage study tested essential oil control candidates at two different application rates and indicated that the treatment consisting of the highest concentration of cedarwood oil (5.2L/ha) and the lowest concentration of rosemary oil (0.75L/ha) had the largest effect on grasshopper mortality; cedarwood at 10% reduction and rosemary oil at 6% reduction. However, again, these results were not statistically significant. The two studies that were conducted in the field did not use different application rates for each plant extract and the rates used were determined based on market cost for commonly used grasshopper control methods. Thus, although the results of the tests presented in this thesis do not indicate adequate efficacy of the agents in their current forms, there is still potential for further examination of these treatment candidates for grasshopper control. The cage study used only two application rates, and further testing of the effects of the selected plant extracts on grasshopper mortality may show that higher rates or different formulations are needed.

The results of the non-target study (Chapter 4) showed no significant effect of neem oil, cedarwood oil or carbaryl bait (Ecobait™) on carabids or phalangids. Cedarwood oil had the greatest effect on carabids compared to other treatments, but only with a 2% mean reduction in abundance after treatment. For

phalangiids, neem oil had the greatest effect, with a 21% mean reduction after treatment. Mean numbers of carabids and phalangiids increased in all other treatments and the control after application. These results support the use of carbaryl bait as treatment for grasshoppers, however, large variability in the results, and the fact that only two arthropod families were studied indicate potential for a more in-depth, longer term study to determine the effects of carbaryl bait on non-target organisms. The GIS maps, representing the spatial distribution of grasshopper abundance at JCP, showed a large variation in spatial patterns, both over the season, as well as between years. However, a region in the north section appears to have had elevated numbers of grasshoppers throughout time. Therefore, it is important to investigate the potential cause, such as environmental conditions (e.g. vegetation, soil characteristics, slope and aspect, moisture, etc.) for this concentration of grasshoppers.

Recommendations for further research

Recommendations for further study of the effects of these plant extracts on grasshoppers include:

- altering application rates to include higher concentrations
- testing different formulation of treatments (e.g. microencapsulation or addition of adjuvants and other agents to enable volatile oils longer persistence)

- adjusting the timing of treatments (based on temperature and/or insect development) e.g.: applying treatments earlier in the season when grasshoppers are not fully developed and before they are able to fly may make them more vulnerable to mortality resulting from contact with the essential oils. Also, the higher the air temperature, the more volatile, and less effective the essential oils are, therefore, treatment applied earlier in the year, as well as early in the morning could have a dual increase in effectiveness
- collecting more detailed data on behaviour and development (i.e. sublethal effects) of treated versus untreated insects (e.g. moulting, feeding, and reproductive behaviour)
- testing for non-target effects as pesticide development continues, in support of registration requirements and sustainable use.

Application of GIS technology to forecast grasshopper outbreaks discussed in Chapter 5 may provide another way to effectively assist in controlling grasshopper damage to fields. Grasshopper infestations occur when isolated populations expand and spread in response to suitable environmental conditions and changes in rates of reproduction and survival (Johnson *et al.* 1996). By collecting grasshopper density data and incorporating weather data and knowledge of grasshopper life cycles into infestation forecasts, land managers can stay more informed about the likelihood of the economic threat to their

crops. Using a GIS to map grasshopper abundance in order to determine areas of high infestations would allow landowners to focus their control measures in areas that require treatment and apply treatments under conditions where they would be most effective. An example of this is the Alberta Grasshopper Forecast which is compiled yearly for the province of Alberta by Alberta Agriculture, Food and Rural Development (AAFRD 2005), but could be more effectively applied in smaller areas using more data points. This would be economically beneficial to land managers by avoiding application of unnecessary pesticide treatments and reduce potential for harm to the environment.

Recommendations for DUC/JCP grasshopper management plan

This master's research was partially funded by Ducks Unlimited Canada (DUC), a conservation organisation that is interested in contributing to the development of pesticides that reduce the threat to ecosystem health. Part of the purpose of this project was to assist in developing a grasshopper management plan to be used at Jefferson Community Pasture (JCP). While the results of the experiments on plant extracts for grasshopper control do not currently offer a viable solution for pest control at JCP, the following paragraphs consider measures that can be applied in order to manage grasshoppers in an ecologically protected habitat.

Carbaryl bait (Ecobait™) was used to control grasshopper infestations at JCP in the summer of 2002 and 2003. Grasshopper abundance was highest at roadsides

and field edges and was, therefore, applied predominantly in these areas. Because bait treatment has lower concentrations of active ingredients and reduced rate of drift than that of common-use pesticides that are applied using spray formulations (Johnson & Henry 1987), this is a less environmentally threatening grasshopper control method for land managers. Additionally, George *et al.* (1992) state that carbaryl bait is less toxic to birds than pesticide sprays because the threat of exposure from inhalation or contact with the pesticide is eliminated. While this control method uses carbaryl, a synthetic pesticide, the threat to avian and other animals is reduced and, therefore, should be considered an option for DUC managers until a more ecologically sustainable alternative is available.

Biological control is another method of grasshopper control that should be considered by land managers interested in reducing synthetic pesticides that are introduced into the environment. Lomer *et al.* (2001) reports that isolates of two fungi (*Beauveria bassiana* and *Metarhizium anisopliae* var. *acridum*) being developed for grasshopper and locust control could offer pest control alternatives with minimal environmental impact. According to Lomer *et al.* (1999), the advantages of using *Metarhizium anisopliae* for grasshopper control are as follows: efficacy and persistence, low vertebrate toxicity, little environmental impact, conservation of natural enemies and potential for recycling. Johnson *et al.* (2002) found that neither *Beauveria bassiana* nor *Metarhizium anisopliae* var. *acridum* had

significant negative effects on growth and development of ring-necked pheasants when fed grasshoppers infected with these fungi. As biocontrol methods are being developed for use in Canada, application of entomopathogens in a grasshopper management program proposes another less environmentally costly option for managers.

Integrated pest management (IPM) uses a combination of control methods to enable land managers to tailor their pest control efforts to meet the demands of particular situations. Because grasshoppers are a food source for birds and other animals, and because repeated application of synthetic pesticides can have harmful effects on many non-target organisms, integrated pest management has become an important concept for landowners that wish to control infestations without disrupting ecosystem function. Use of GIS software, biocontrol measures, low toxicity pesticides and applying knowledge of grasshopper life cycles to control outbreaks may provide land managers, such as DUC, with a pest management program that poses minimal threat to the ecosystem. By identifying areas where grasshoppers are most abundant, forecasting population increases, and understanding development of grasshopper populations, DUC personnel can apply pesticide treatments only in areas where grasshoppers are most threatening to crops. This can be achieved by systematically collecting point data of grasshopper densities and age structure at JCP. By using these data as input in a GIS, and taking into consideration past, present and forecasted

weather, managers can make informed predictions about where grasshoppers will pose the greatest threat. Using carbaryl bait treatments, entomopathogens, or a combination of treatments, such as plant extracts, if they are further developed and targeted, can be decided upon based on how severe the pest problem, predicted duration of grasshopper infestations and other factors that may be important, for example proximity to water, economic resources, life cycles of other wildlife that use the area.

Development of an IPM program for JCP requires informed decisions regarding abundance and location of grasshopper populations, environmental effects of pesticides that are available (non-target effects and effectiveness of control candidates on grasshoppers), and cost of the various management tools that are available. This master's research was intended to provide DUC managers with information to assist in making these decisions.

Conclusions

Development of new pest control methods involves applying control candidates at known rates and then determining if there is any effect on mortality or behaviour of pest species. If an effect is evident, then application rates and methods can be altered to find formulations that are effective at a level necessary to control infestations and that are economically feasible. Pesticide development should also include testing control candidates on non-target organisms to

determine if pesticide applications will have a broader effect than desired. This development is a step-wise process and the research presented here is the first step in determining if the selected plant extracts showed potential for grasshopper control and did not pose a significant threat to two non-target arthropods.

Previous experiments indicate that essential oils are active against pests in enclosed conditions, however, when applied in a field setting to control grasshopper pests, no significant effects were found. The non-target tests indicate no negative effects of plant extracts on carabids and phalangiids, therefore, more testing of essential oils and other alternative, less environmentally harmful pest control methods is necessary to ensure sustainable development of agriculture and other land use. The methodology and multi-faceted approach used in this research is important for development of pest control candidates. Testing of the selected essential oils for control of grasshoppers done here was novel and preliminary, therefore, these control candidates should not be discounted. Other studies using plant extracts for pest control have shown positive results and further study of these alternative methods for controlling grasshopper pests is promising as sustainable control of grasshoppers is a desirable result given the serious damage that these pests have caused to crops in the Canadian Prairie provinces.

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