

**STUDIES ON PYRETHROID EAR TAGS AND PYRETHROID RESISTANCE
IN HORN FLY POPULATIONS IN MANITOBA**

BY

FELIX SIMASIKU MWANGALA

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**Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements
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ABSTRACT**Studies on Pyrethroid Ear Tags and Pyrethroid Resistance
in Horn Fly Populations in Manitoba.****By****Felix Simasiku Mwangala****Major Advisor: Dr. Terry D. Galloway.**

This study was undertaken from 1987 to 1990, in Manitoba. A producer survey was conducted in 1987 to determine the extent of insecticidal ear tag usage in Manitoba. More than 80% of the 200 cattle producers who responded to the questionnaire applied tags to control flies. Ear tags were more rapidly adopted by beef producers than by dairy producers. Most of the producers applied one tag per animal and were satisfied with the control afforded by ear tags. Fenvalerate tags were used extensively followed by permethrin tags.

Field horn fly populations were evaluated for resistance to fenvalerate and permethrin using the impregnated filter paper petri-dish method in 1987 to 1989. Resistance factors were calculated by comparison to a susceptible laboratory strain from Kerrville, Texas. The number of resistant populations and intensity of resistance increased during the study. Resistance factors ranged from 0.11- to 5.5-fold and 0.6- to 14-fold in 1987, 0.03- to 38-fold and 0.1 to over 100-fold in 1988, and 1.0- to 62-fold and 0.8- to over 100-fold in 1989, for fenvalerate and

permethrin, respectively. Resistant flies were sampled on 3 herds in 1987, 6 herds in 1988, and 10 herds in 1989.

Fenvalerate and permethrin residues on hair of cattle at Glenlea Research Station were determined, using gas chromatography, over a 3 month period following application of two tags per animal or one tag for every other animal. Cattle with two tags had significantly higher residues on the head and neck region for one month and on the body and rump for 2 wk than cattle with one tag or without a tag in the same herd. Residues on cattle with or without a tag in the same herd were similar. Residues declined by 80 to 86% on the head, 73 to 78% on the body and 36 to 84% on the rump. The isomeric composition varied from 51-61% SR,RS:39-49%SS,RR for fenvalerate and 61-67% *trans*:33-39% *cis* for permethrin.

Fly numbers on cattle with two tags per animal and one tag for every animal were similar in 1987 and 1988. Fly numbers increased towards the end of the season and were higher in 1988 than in 1987. The LC_{50} 's of these flies increased during each season but the LC_{50} 's declined between fall and spring. Susceptible flies were eliminated from the population upon application of tags.

Stability of resistance to pyrethroids was assessed in two resistant horn fly populations at Glenlea and Libau Community Pasture in 1989. The response of these populations to fenvalerate and permethrin was similar during

the season in the absence of tags. Tag formulations containing 20% diazinon, 4% cypermethrin + 18% diazinon, 10% λ -cyhalothrin, and 10 λ -cyhalothrin + 13% piperonyl butoxide were effective against pyrethroid resistant horn flies; and combination tags were as effective as single formulation tags.

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CHAPTER I

INTRODUCTION

The Problem

The horn fly, *Haematobia irritans* (L.), is an obligate blood sucking ectoparasite of cattle. It was introduced into the United States (U.S.) from Europe about 1885 (Riley 1889). In Canada, the fly was first reported in Oshawa, Ontario in 1892 (Fletcher 1892) and established itself as a major cattle pest in all provinces of Canada by 1900 (Hearle 1938). Horn flies cause over US \$730 million (Drummond 1987) and Canadian \$30 million (Skaptason 1950) losses to cattle production in the U.S.A. and Canada, respectively.

The traditional and most effective method of controlling horn flies has been the application of insecticides on cattle. This strategy has been effective because horn flies rest on cattle most of the time. Various delivery methods have been used to deposit insecticides on cattle hair. These methods include spraying, dusting and self-treatment devices, including backrubbers, dust bags, oilers and, more recently, insecticide-impregnated ear tags.

Insecticidal ear tags were introduced in the late 1970's and gained widespread and rapid acceptance by producers. Unlike other insecticide application methods that required regular handling of cattle and/or maintenance of equipment, insecticidal ear tags provided a simple, easy

and effective season long control method for susceptible flies. Because of these properties, ear tags became very popular with producers.

However, within 2 to 4 yr of widespread ear tag usage, horn flies developed resistance to insecticides in the ear tags in the U.S. (Kunz and Schmidt 1985a; Quisenberry *et al.* 1984; Schmidt *et al.* 1985; Sheppard 1984). In Canada, ear tags were registered in 1981 (S. Van Walleggem, Ag. Canada, pers. comm.) but prior to the start of this research, there were no confirmed reports of horn fly resistance to insecticides in ear tags.

Objectives of the Research

The objectives of this research were to determine:

- a) the extent of insecticidal ear tag usage in Manitoba,
- b) the susceptibility of horn flies in Manitoba to pyrethroids,
- c) the levels of pyrethroid residues deposited on hair over various regions of cattle following application of ear tags,
- d) the changes in susceptibility of horn flies from cattle treated with fenvalerate and permethrin tags during the season,
- e) the stability of pyrethroid resistance in field populations of horn flies in the absence of selection

pressure, and

f) the efficacy of alternative control strategies against pyrethroid-resistant strains.

Thesis Organization

The results reported in this thesis are from experiments carried out from September, 1986 to April, 1990. Extent of insecticidal ear tag usage in Manitoba was determined through a questionnaire sent to producers in April, 1987. The questionnaire was also used to identify potential producer co-operators for future studies. Horn flies used in the susceptibility studies were collected from producers' cattle in various regions of the province during the summers of 1987, 1988 and 1989. Collection of hair samples for pyrethroid residue analysis and changes in the susceptibility of horn flies from cattle tagged with permethrin and fenvalerate tags were carried out at the University of Manitoba's Glenlea Research Station in the summers of 1987 and 1988. Studies on the stability of pyrethroid resistance in horn flies were conducted at the Glenlea Research Station and Libau Community Pasture in the summer of 1989. Efficacy of alternative control strategies against pyrethroid-resistant horn flies was evaluated on cattle at Libau Community Pasture, Narcisse Community Pasture, Gunton Bull Test Station, and cattle of producers near Libau and Lundar.

This thesis is divided into Chapters, each covering a separate aspect of the research. The problem and objectives of the research are outlined in Chapter I. Literature pertinent to the research is reviewed in Chapter II. Results of the experimental studies are described in Chapter III, and are prepared in sections in a style suitable for publication as a series of scientific papers. Section I covers the extent of ear tag usage in Manitoba. Susceptibility levels of horn flies to pyrethroids in Manitoba are reported in Section II. Distribution of pyrethroid residues on hair of cattle following application of ear tags are reported in Section III. Section IV covers studies on the dynamics of horn fly resistance to pyrethroids on tagged cattle during the season. Studies on the stability of pyrethroid resistance in horn flies in the absence of selection pressure are reported in Section V, while the studies on the efficacy of other control strategies against resistant strains are covered in section VI. A general discussion and conclusions from the research are presented in Chapters IV and V, respectively.

CHAPTER II

PERTINENT LITERATURE

Introduction

The horn fly, *Haematobia irritans* (L.), is an obligate blood sucking ectoparasite of cattle and is distributed throughout most of Europe, North Africa, Asia Minor, and the Americas (McLintock and Depner 1954). It was introduced into the United States from Southern Europe in about 1885, probably through shipments of cattle from Europe (Riley 1889). The fly became established in the New World and is now distributed from Venezuela in the south to about latitude 53° 33' North (McLintock and Depner 1954). In Canada, the fly was first reported in Oshawa, Ontario in 1892 (Fletcher 1892) and was established as a major cattle pest in all the provinces by 1900 (Hearle 1938).

Biology of Horn flies

The horn fly appears on cattle in spring with the onset of warm weather. On emergence, the flies seek their hosts and are attracted by carbon dioxide, heat, colour, and odour (Dalton et al. 1978). The latter seems to be the major attractant, at least over short distances (Dalton et al. 1978). The flies are also more attracted to dark coloured animals than to light coloured ones (Burns et al. 1962) and seem to prefer a temperature of about 37.8 °C (Dalton et al.

1978). Horn flies can disperse long distance over short periods (Beadles et al. 1975; Byford et al. 1987a; Eddy et al. 1962; Kinzer and Reeves 1974; Kunz et al. 1983; Skoda et al. 1987; Tugwell et al. 1966). Flies can disperse 12 km in 10 hr (Kinzer and Reeves 1974) and up to 26 km (Beadles et al. 1975). Newly emerged flies seeking their first hosts are the primary dispersers (Chamberlain 1982; Guillot et al. 1988; Tugwell et al. 1966). Once hosts are located, there appears to be little propensity for dispersal (Chamberlain 1981, 1982; McLintock and Depner 1954).

The flies spend most of their time resting on the host. They are intermittent feeders and take 24 to 36 blood meals per day (Harris et al. 1974). The flies rest on parts of the host that cannot be reached by the head or tail, slightly behind the shoulders, at the base of the horns, on the middle of the belly and low on the hind legs (McLintock and Depner 1954). Horn flies are sensitive to changes in temperature and move to the most favourable locations (Hammer 1942). When the weather is cloudy or cool, the flies always rest on the back of cattle, but when the weather is bright and sunny, their resting sites depend on air temperature. At temperatures up to 19 °C, flies tend to congregate on the back, but at temperatures of 26 to 29 °C, flies congregate on the shaded regions underneath the belly, on lower parts of the hind legs and on the scrotum or the udder (McLintock and Depner 1954).

Female flies leave the host for short periods to oviposit in fresh dung pats. The flies lay their eggs chiefly, if not exclusively, in the dung (McLintock and Depner 1954). The dung is most attractive to the flies during the first 2 minutes after defecation (Sanders and Dobson 1969). The eggs are deposited in groups of four to six on the pat surface, along the sides or in close proximity to the pat. Oviposition occurs during the day and night, but more adults are produced in the dung pats deposited at night than those deposited during the day (Kunz et al. 1970). This may be due to slow drying of the dung pats at night which increases egg survival. The number of eggs laid by a single female at each laying depends on temperature and humidity, and can be up to 24 (Bruce 1940). Each female is capable of laying 375 to 400 eggs over her life span (Bruce 1942), with maximum egg production on the third day after maturity (Palmer and Bay 1982).

Eggs hatch within 24 hr and viability depends on the temperature and humidity. The optimum temperature for egg development is about 20 °C. Above this temperature, survival of eggs decreases (Melvin 1934). The eggs also require high humidity to avoid desiccation.

Upon hatching the larvae burrow into the dung, where they feed. The larvae go through three instars and developmental time depends on temperature. Pupation occurs in the lower part of the dung or in the ground immediately

beneath it and may take 3 to 12 days (McLintock and Depner 1954). At 29 °C, adults emerge in three to four days (McLintock and Depner 1954). Adult flies can survive for seven weeks in the field (Bruce 1942). In the laboratory, horn flies have been kept on artificial diets or on a calf for as long as 28 days (McLintock and Depner 1954). Under hot, humid conditions, one generation from egg to adult may take only 8 to 14 days and up to 30 days during spring and fall (Depner 1961). In warmer regions, the generation time of horn flies is about 8 to 10 days (Kunz et al. 1970), and the season may be 8 to 12 months (Kunz and Cunningham 1977; Thomas and Kunz 1986; Thomas et al. 1987). In Manitoba and most of Canada, the generation time is more than 14 days and the season is less than 5 months (Depner 1961; McIntock and Depner 1954).

Diapause occurs mainly in the pupal stage (Bruce 1940; Hammer 1942) and the intrapuparial pharate stage (Thomas 1985), though McIntock and Depner (1954) reported overwintering third instar larvae. Diapause is mainly triggered when adults are exposed to short photoperiod and the larvae are exposed to cool temperatures during fall (Thomas and Kunz 1986; Wright 1970).

Economic Impact of Horn Flies

Horn flies cause an estimated \$730 million (U.S.) and \$30 million (Canadian) loss annually to beef cattle and

dairy industries of the United States and Canada respectively (Drummond et al. 1981; Skaptason 1950). These losses arise more from morbidity than mortality. Morbidity losses are principally from irritation and annoyance. Cattle under attack from horn flies are restless, nervous and difficult to handle. They lash their tails, swing their heads from side to side, and kick at their bellies; this results in unnecessary expenditure of energy and reduced grazing time.

The effects of horn control on beef and milk production have been reviewed by Palmer and Bay (1981), Drummond et al. (1981), and Drummond (1987). A number of studies have been conducted to quantify the effect of horn flies on the performance of cattle. The results of these studies have varied, probably due to the influence of other factors, such as nutrition and pasture quality, breed differences, presence of other insects, and horn fly infestation. Kinzer et al. (1984) reported a reduced average daily weight gain of 0.045 kg to 0.162 kg in steers exposed to horn flies in screened pens. They also observed a reduction in feed efficiency; fly-infested steers required 9.1 to 24.3% more feed to achieve weight gain equal to the screened ones. Yearling steers that were protected from horn flies on pasture gained an average of 8 kg more than the unprotected steers over the season (Harvey and Brethour 1979). An increase in live weight gain of 17.7% was also reported by

Haufe (1982) in steers protected by fenvalerate ear tags over a 115-day grazing period on range. However, in a 3 yr study, Schreiber et al. (1987) reported no significant cow, calf or cow/calf weight gains when cows were tagged with one fenvalerate tag per head. Brethour et al. (1987) did not observe any significant returns in using flucythrinate tags on Brahman x Hereford yearling heifers and very little on Angus x Hereford heifers.

Loss in milk production attributed to horn flies has been estimated at \$59 million (U.S.) per year (Anonymous 1976). Morgan and Bailie (1980) found a mean increase of 0.8 to 1.0 kg in milk yield per cow per day from horn fly free cows. An increase of 20% to 25% in milk production was also reported by Skaptason (1948). Reduction in milk yield has been measured indirectly using the weaning weights of calves. Calves weaned from cows that were protected from horn flies had an average weaning weight of 5.8 kg/calf more than those weaned from untreated cows (Campbell 1976). Kunz et al. (1984) demonstrated that calves from fenvalerate-treated cows outweighed calves from untreated cows by 7.4 kg per head at weaning which gave an increase of up to \$8.38 per \$1 spent on fly control. Similar results have been reported by Kopp et al. (1984) and Quisenberry and Strohbehn (1984) in North Dakota and Iowa, respectively. In a study by Cocke et al. (1990a), male and female calves gained 6.7 and 11.3 kg, more than males and females from an untreated

herd, representing a \$5.00 to \$ 7.44 return for each \$1.00 spent on ear tags. However, when the data were analyzed by breed, some breeds did not show any significant gains. Fly control did not affect calf weight gain in a study by Essing and Pund (1965).

Horn flies also cause blood loss in their hosts. A cow carrying 500 flies loses about 7 mL of blood per day (Harris and Frazar 1970). In addition, cattle attacked by horn flies have higher heart rates, respiration rates and rectal temperatures than the horn fly free ones (Schwinghammer et al. 1986).

Horn flies feed intermittently and move frequently from one animal to another. They are potential vectors of disease agents e.g. the parasitic filarial worm (*Stephanofilaria stilesi* Chitwood), which causes dermatitis (Hibler 1966). Butler et al. (1977) demonstrated the capacity of horn flies to transmit disease agents mechanically by using radioisotope ^{32}P . The flies transferred ^{32}P to a new food source in their first and second feeding, and the saliva was labelled for about two hours.

Attempts have been made to relate horn fly populations to reductions in potential production in order to establish economic threshold levels. Different economic thresholds have been determined for different localities. Haufe (1981) estimated the economic threshold at 12 flies per animal. In

Texas, the economic threshold is 250 flies per animal (Patrick et al. 1989, cited in Cocke et al. 1990a).

Control Methods

Horn flies spend most of the time on the host, and are therefore highly susceptible to insecticides applied to the host using a variety of methods. The methods include dipping, spraying, dusting, ultra low volume aerial spraying, and self-application devices.

The commonly used early methods were dipping and spraying, and to a lesser extent dusting. These methods had certain disadvantages. Dipping involved rounding up the cattle from pastures to dipping vats containing insecticide wash. Dipping required a fairly large labour input to round up cattle, to force them into the dip vat and attend to them as they swim through the vat. Spraying also involves rounding up cattle from pastures and herding them into chutes where they are confined and sprayed. Cattle can also be sprayed on pasture using vehicle-mounted sprayers. Dusts may also be applied to confined cattle either with hand dusters or with power dusters. Most of the insecticides that were used with these methods were not persistent and protected animals for less than two weeks. Therefore, insecticides had to be applied fortnightly to achieve good control. In addition to the labour required to round up cattle, spraying and dipping resulted in physical stress and

interrupted grazing. As the cost of labour increased, spraying and dipping fell out of favour and producers started to use self-application devices.

Ultra low volume aerial spraying was used for horn fly control; low volumes of insecticides were sprayed on cattle on pastures by low flying aircraft. Ultra low volume spraying did not require gathering of animals and utilized low volumes of insecticides which resulted in lower residues. However, the noise from the aircraft excited cattle and more applications were required per season because the insecticides were not persistent. This made U.L.V. aerial spraying expensive and impractical under range conditions (Kinzer 1969).

Self-application devices include backrubbers, oilers, dust bags and, more recently, insecticide-impregnated ear tags. With these methods, there is no need to round up cattle regularly, therefore, less labour is required. Backrubbers are made of burlap or canvas wrapped around barbed wire or chains which are strung between posts. The burlap or canvas is saturated with an insecticide. The backrubbers and oilers are based on the principle that cattle like to rub themselves against posts and other objects. Cattle treat themselves when they rub against backrubbers and oilers. Backrubbers have the disadvantage that they have to be re-saturated regularly for effective control because insecticide formulations are subject to

evaporation. In addition, the effectiveness of backrubbers and oilers is reduced when there are trees in pastures that can be used as alternatives. Dust bags are made of burlap and contain inner liners that regulate dust flow and prevent wetting of the dust by rain. The bags are suspended at about the height of the withers in either free choice or forced use situations where cattle are forced to use them to get to water or minerals or pass from one pasture to another. Dust bags require less attention than backrubbers once they are established (Lancaster and Simco 1967). The major problems with dust bags are compaction of the dust within the bag and wetting by rain. Therefore, a dust with good flowability should be used, and dust bags should be protected from rain.

The major disadvantage with backrubbers, oilers and dust bags is that they have to be placed at points where cattle congregate. When rotational grazing is practised, backrubbers, oilers and dust bag stations must be established in each pasture or forced use systems have to be established.

Most ear tags are made from polyvinyl chloride mixed with a plasticizer, and impregnated with an insecticide. Ear tags are designed to release the pesticide slowly over a prolonged period. The earlier ear tags were impregnated with organophosphorus insecticides but these fell out of favour because they were effective for only a short period

(Ahrens 1977; Harvey and Brethour 1970). They were replaced by pyrethroid-impregnated ear tags which provided season-long control of horn flies (Ahrens and Cocke 1979). Ahrens and Cocke (1979) obtained a 100% reduction in horn flies within 24 hr. Control was maintained for 20 weeks with two fenvalerate tags per head. Ninety-five per cent control of horn flies was achieved with one permethrin tag per head over a period of 17 weeks (Williams and Westby 1982). Good control of horn flies can also be achieved by partial herd treatment. Untagged animals kept in the same pasture with cattle treated with two fenvalerate tags per head were completely free from horn flies for 20 weeks (Ahrens and Cocke 1979). Fenvalerate-impregnated ear tags provided complete horn fly control in a herd when their nursing calves were tagged in one ear only (Harvey and Brethour 1983). In addition, Knapp (1982) obtained complete horn fly control for two seasons when the tags were left on cattle.

Pyrethroid-impregnated ear tags have been rapidly accepted by producers and widely used because of their effectiveness. Tagging does not require additional specialized application equipment and labour. Tags can be applied when animals are being dewormed or vaccinated, before going to the pasture.

Mode and Rate of Release of Pyrethroids from Ear Tags

The mode and rate of release of pyrethroids from ear tags and the spread of residues on cattle are still unclear. Pyrethroids are broad spectrum contact insecticides that have low mammalian toxicity, high insecticidal activity and low environmental persistence (Elliott et al. 1978). They also have low vapour pressure, poor water solubility and a strong tendency to adsorb onto surfaces, especially to lipophilic surfaces (Miller and Adams 1982; Ruigt 1985). Pyrethroids also exhibit a repellent effect and act as anti-feeding agents to many insect species and Acarina (Hall 1979; Penman et al. 1981; Shemanchuk 1981). Permethrin does not repel horn flies but is an irritant. Horn flies will land on cattle treated with permethrin but the flies do not rest or feed on them (Khan and Colwell 1984).

The rate of release of pyrethroids from an ear tag follows a parabolic curve (Miller et al. 1983a, 1986). More insecticide is released immediately after tags are installed and the rate of release decreases as the season progresses. The amount of fenvalerate released by an 8% fenvalerate ear tag declines from 3.9 mg per day at day 10, to 0.9 mg per day at day 180, while that of a 10% permethrin ear tag declines from 6.5 mg per day at 10 days to 1.5 mg per day after 180 days (Miller et al. 1983a). Furthermore, not all of the insecticide in the ear tag is released during the fly season. More than 50% of the insecticide remains in the tag

after 115 to 180 days following tag application (Haufe 1982; Miller et al. 1983a).

The rate of release is influenced by environmental conditions and the formulation of the tag. Pyrethroid diffuses from ear tags at a higher rate in warm weather than in cold weather (Miller et al. 1986; Taylor et al. 1985). The plasticizer in the ear tag also influences the amount of insecticide released in the season. Fenvalerate ear tags, which contain dioctyl adipate (DOA) as the plasticizer, release about 25% of the insecticide during the season (Haufe 1982; Miller et al. 1983a). Permethrin ear tags, with dioctyl phthalate (DOP) as the plasticizer, release about 45% of the insecticide (Miller et al. 1983a).

The insecticide that is released from the ear tag spreads over the animal's coat by diffusion through sebaceous secretions and direct contact through grooming (Beadles et al. 1977; Taylor et al. 1985). The concentration of the insecticide deposited on the hair of cattle is not uniform. Taylor et al. (1985) found higher concentrations of cypermethrin on the hair from the flanks, farther away from the tags than other areas sampled. Holbrook (1986) found higher concentrations of fenvalerate on the hair near the ears than from other regions of the body.

Development of Insecticide Resistance

Resistance has been defined as "the development of an ability in a strain of insects to tolerate doses of toxicants which would prove lethal to the majority of individuals in a normal population of the same species" (Anonymous 1957). Resistance is an evolutionary phenomenon, brought about by intensive selection of the insect population by application of insecticides. Resistance is considered to develop through three stages (Comins 1984). The first precedes the use of the insecticide. Before an insecticide is applied to a pest population, the population is usually susceptible, and resistance genes occur at low frequencies in the population (Crow 1957; Oppenoorth and Welling 1976). The resistance genes are usually thought to be disadvantageous in an environment without insecticides and during the early stages of resistance development, hence the reversion to susceptibility observed when insecticides are withdrawn (Abedi and Brown 1960; Crow 1957). Resistance genes arise in populations because of recurrent mutations and are maintained at low frequency by a balance between mutation and natural selection (Georghiou 1972).

The second stage starts when the insecticide is applied. Selection for individuals carrying the resistance genes that are adapted to survive in the insecticide-contaminated environments begins. The frequency of resistance genes in the population increases though they are

still below the level at which control efficiency is noticeably reduced. At this stage, the resistance genes occur mainly in heterozygous individuals (Roush and McKenzie 1987). Reconstruction of the genetic background occurs in which the resistance genes are integrated with other fitness factors (Wood and Bishop 1981). With continued selection, the resistance genes appear in the homozygous state and may be fully incorporated into the genetic background of the population. Resistance management strategies are best applied during this stage before individuals carrying the resistance genes become prevalent. In the last stage, control failures are noticed and resistance is detected by susceptibility tests. At this stage the only option is to increase dosage or abandon the insecticide.

History of Horn fly Resistance to Insecticides

There is very little information on horn fly resistance to insecticides in Canada. The information presented in this section is mainly from studies done in the U.S..

The first organic insecticides to be used widely for horn fly control were chlorinated hydrocarbons (DDT, toxaphene and methoxychlor) (Laake 1946; Matthysse 1946). Resistance to these insecticides was suspected in the late 1950's (Burns et al. 1959; Lewis and Eddy 1961; McDuffie 1960). Subsequently, resistance to toxaphene was confirmed in 1960 (Harris 1964; McDuffie 1960) and DDT in 1961 (Harris

1964). Resistance to methoxychlor was confirmed in 1965 (Burns and Wilson 1967, cited in Sparks *et al.* 1985). Organophosphorus (fenchlorphos, crufomate, malathion) insecticides and a carbamate (carbaryl) were recommended for use from 1960 to 1965. Resistance to fenchlorphos was reported in 1962 following intensive use in backrubbers (Burns and Wilson 1963).

Insecticidal ear tags were introduced in the late 1970's and early 1980's. Resistance to the first organophosphorus insecticide (stirofos) used in ear tags was documented in 1983 after two seasons of use (Sheppard 1983). Pyrethroid-impregnated ear tags followed organophosphorus tags. Fenvalerate and permethrin were the first pyrethroids to be used. Resistance to these pyrethroids was reported after 2 to 4 years of use (Kunz and Schmidt 1985a; Quisenberry *et al.* 1984; Schmidt *et al.* 1985; Sheppard 1984). Resistance to these two pyrethroids conferred cross-resistance to other pyrethroids, including cypermethrin, deltamethrin and flucythrinate, as well as DDT and methoxychlor (Byford *et al.* 1985; Byford and Sparks 1987; Sheppard 1984).

Resistance to pyrethroids following tag use developed rapidly and has been the most widespread among horn fly populations in North America. Resistance to pyrethroids was first reported in 1982 in Florida (Kunz and Schmidt 1985a), then resistance progressed in a northwesterly fashion to

Georgia, Alabama, Mississippi and Louisiana. By the end of 1984, resistance was either confirmed or strongly suspected in the above states, plus Oklahoma, Kansas, Kentucky, Texas, Nebraska, Tennessee, Arkansas, California and Hawaii (Kunz and Schmidt 1985a). By the end of 1987, resistant horn fly populations were reported in 24 states in the southeastern and midwestern U.S., as well as California, Hawaii, and Oregon (Marchiondo 1987). Pyrethroid resistance was reported in North Dakota in 1986 (Meyer and Kopp 1986).

Mechanism of Resistance to Pyrethroids in Horn Flies

Resistance to pyrethroids in horn flies is due to a number of factors which are physiological, biochemical and behavioural in nature. The major resistance factor is consistent with the insensitive target site (*kdr*) type of resistance (Bull et al. 1988; Byford et al. 1985; McDonald and Schmidt 1987; Roush et al. 1986). The *kdr* factor confers cross-resistance to most pyrethroids, DDT and methoxychlor but not to carbamates or organophosphorus insecticides (Bull et al. 1988; Byford et al. 1985, 1988; Byford and Sparks 1987; Sparks and Byford 1988). The resistance conferred low cross-resistance to cyhalothrin (Sparks and Byford 1988).

Increased metabolism of pyrethroids in resistant horn flies has been demonstrated in studies using synergists, which inhibit metabolic enzymes (Bull et al. 1988; Byford et

al. 1985; McDonald and Schmidt 1987; Sparks and Byford 1988). Enhanced metabolic detoxification appears to contribute more to resistance during the later stages in the development of resistance than at earlier stages (Bull et al. 1988). However, the levels of synergism observed in pyrethroid-resistant strains are usually too low to account for higher levels of resistance in the strains (Bull et al. 1988; Byford et al. 1985; McDonald and Schmidt 1987). Thus the contribution of increased metabolism to pyrethroid resistance in horn flies is minor.

Behavioural adaptations might also contribute to resistance in horn flies (Byford et al. 1987b; Lockwood et al. 1985; Quisenberry et al. 1984). Stimulus-dependent behavioural resistance was demonstrated in the laboratory, where pyrethroid-resistant horn flies were more easily irritated and/or repelled by fenvalerate and permethrin than susceptible flies (Lockwood et al. 1985; Quisenberry et al. 1984) and in the field, where resistant horn flies preferred areas with low residues on tagged cattle (Byford et al. 1987b). The imbalance in the distribution of resistant horn flies was not observed on untagged cattle that were isolated from the tagged herd (Byford et al. 1987b). However, behavioural resistance cannot be an effective mechanism on its own because flies may eventually acquire a lethal dose.

Factors Influencing the Development of Resistance in Horn Flies

Factors that are known to influence the rate at which resistance develops in field populations have been grouped into genetic, biological or ecological and operational categories (Georghiou and Taylor 1976, 1977a, b). Operational factors relate to the way insecticides are used and can be manipulated to forestall the development of resistance. Most of the genetic and biological factors are beyond man's control. The influence of these factors on the development of resistance have been studied using computer simulation models (Comins 1977; Curtis et al. 1978; Georghiou and Taylor 1977a, b; Mani and Wood 1984; Tabashnik and Croft 1982; Taylor and Georghiou 1979, 1982; Taylor et al. 1983). Sparks et al. (1985) reviewed the factors that contributed to the rapid development of pyrethroid resistance in horn flies and concluded that the ear tag-horn fly combination met most of the conditions that lead to rapid development of resistance.

The major factors responsible for the rapid development of resistance in horn flies are initial frequency of resistance genes, dominance of the resistance gene, persistent residues and some biological attributes of horn flies such as monophagy, short generation time and multivoltinism. These factors interact in their influence on resistance development.

Resistance to DDT in horn flies (Byford et al. 1985; Harris 1964) has been implicated in the rapid development of pyrethroid resistance in horn flies (Sparks et al. 1985). Use of DDT in the 1950's might have predisposed horn flies to pyrethroids through the selection of the *kdr* mechanism. In Danish house fly populations, the *kdr* gene persisted for more than 20 years (Keiding 1977). It is possible that the *kdr* gene already occurred at high frequencies in some horn fly populations when pyrethroid tags were introduced.

The dominance of a resistance gene is related to the resemblance of the heterozygous offspring to one of the parents (Roush and McKenzie 1987). The resistance gene is dominant when the heterozygotes resemble the resistant homozygous parents, and recessive when the heterozygotes resemble the susceptible homozygotes. In a treated population, expression of dominance is related to dose of insecticide applied (Curtis et al. 1978; Taylor and Georghiou 1979). If a high dose that is lethal to both heterozygous and susceptible individuals is applied, then the resistance gene is rendered recessive. A low dose that kills only the susceptible homozygotes, renders the resistance gene dominant. Resistance develops more rapidly when it is dominant than when it is recessive (Curtis et al. 1978; Georghiou and Taylor 1977a, b; Roush and McKenzie 1987), because a high dose kills most individuals in the population, leaving only a few homozygous resistant ones.

If the few survivors mate with susceptible immigrants, they produce a population that will be largely comprised of susceptible and heterozygous individuals. These can be killed by the next application at a high dose (Comins 1977; Tabashnik and Croft 1982). It is essential that the few resistant survivors mate with susceptible immigrants for resistance to be delayed (Taylor et al. 1983). However, without immigration of susceptible individuals, as is the case when there are persistent residues, dominance has little influence in the development of resistance (Comins 1977; Tabashnik and Croft 1982; Taylor et al. 1983).

In the case of ear tags, insecticides are released slowly over a prolonged period at a decreasing rate (Miller et al. 1983a, 1986). These residues render the resistance gene recessive at the beginning of the season and dominant at the end of the season. Furthermore, the continuous release of insecticide effectively eliminates immigration of susceptible flies into treated populations, leaving resistant flies to reproduce (McDonald et al. 1987a; Roush et al. 1986). Persistent residues, coupled with the fact that horn flies are obligate ectoparasites of cattle and spend most of the time on the host (McLintock and Depner 1954), ensure that all generations of adult horn flies are selected throughout the season. In addition, many generations of resistant flies are exposed to residues in a season because horn flies have a short generation time (Kunz

et al. 1970; McLintock and Depner 1954), resulting in rapid fixation of the resistance gene into the population. Thus, the persistent but declining residues released from the ear tags appear to be the major factor that has led to rapid development of resistance among the factors known to influence the development of resistance. Dominance of the resistance gene and effectiveness of susceptible immigrants in diluting the resistance gene are determined by residue levels on cattle.

CHAPTER III

SECTION I

**SURVEY FOR INSECTICIDAL EAR TAG USE AMONG CATTLE PRODUCERS
IN MANITOBA, CANADA**

Abstract

A survey was conducted in 1987 to determine the extent of insecticidal ear tag use, tagging rates and levels of control being achieved by beef and dairy producers in Manitoba. More than 80% of producers who responded applied tags to control flies. Of these, 37% applied less than one tag per animal; 59% applied one tag per animal; 2% applied two tags per animal. Eighty-five per cent of beef producers and 70% of dairy producers who responded used tags. More beef producers adopted ear tags earlier than dairy producers did. Sixty per cent of beef producers using tags started using them before 1984 while the remaining 40% adopted them after 1983. On the other hand, 61% of dairy producers using tags began using them after 1983. Eighty-nine per cent of tag-users had used fenvalerate-impregnated ear tags while 38% had used permethrin-impregnated ear tags. Only a small proportion of producers had used tetrachlorvinphos- and cypermethrin-impregnated ear tags (8% and 3% of tag users, respectively). Over 95% of producers applying tags indicated that insecticidal ear tags were effective in controlling horn flies.

Introduction

The horn fly, *Haematobia irritans*, (L.) is one of the major pests of pastured cattle in North America (McLintock and Depner 1954). Horn flies are estimated to cost producers in excess of US\$700 million annually (Drummond et al. 1981) and the need to control them has long been recognized by producers.

Horn flies have been controlled successfully by applying insecticides to cattle. Early control measures against horn flies relied upon frequent applications of insecticides as spray or dust formulations. These methods were labour intensive, and their use declined when self-treating devices such as backrubbers, dust bags and oilers were developed.

In the late 1970's, insecticidal ear tags were developed for fly control on pastured cattle. Ear tags impregnated with pyrethroids gained the most widespread and rapid acceptance by producers because they provided an effective and economic season-long control of horn flies (Ahrens and Cocke 1979; Knapp and Herald 1981; Schmidt and Kunz 1980; Williams and Westby 1980; Williams et al. 1981). Use of the other self-treating devices declined because they required regular servicing and their efficacy was also often inconsistent.

However, within two to four years of widespread ear tag use, there were reports of control failures in the United

States where horn fly populations developed resistance to pyrethroids in ear tags (Kunz and Schmidt 1985a; Meyer and Kopp 1986; Quisenberry et al. 1984; Schmidt et al. 1985; Sheppard 1984). In Canada, ear tags were registered in 1981 (S. Van Wallegghem, Ag. Canada, pers. comm.). The trade names, active ingredient and order of registration of tags in Canada are as follows: Bovaid®, 8% fenvalerate (pyrethroid), 1981; Debantic®, 13.5% tetrachlorvinphos (organophosphorus), 1983; Atroban®, 10% permethrin (pyrethroid), 1984; Gardstar®, 10% permethrin, 1985; Disvap®, 13.7% tetrachlorvinphos, 1985; Austin®, 13.5% tetrachlorvinphos, 1985; Stockaid®, 8% cypermethrin (pyrethroid), 1986; and Guardian®, 7.5% flucythrinate (pyrethroid), 1987. At the time this study was initiated, there were no confirmed reports of control failures due to resistance in Canada. This survey was part of a larger study in which the objective was to investigate horn fly resistance to pyrethroid-impregnated ear tags in Manitoba, and was undertaken to determine the extent of tag use, tagging rates and levels of control being achieved with ear tags in Manitoba. The survey was also used to identify potential producer co-operators for studies on horn fly resistance in Manitoba.

Methods

Five hundred questionnaires (Appendix A), together with self-addressed postage pre-paid envelopes were sent to Livestock Specialists in five agricultural regions of Manitoba (i.e. Interlake, Central, Eastern, Northwest, and Southwest) for distribution to producers in May, 1987 (100 per region). The data are expressed as a percentage of the total number of responses for each particular aspect of the survey.

Results and Discussion

A total of 200 producers responded to the survey representing 40% of the total questionnaires sent to Livestock Specialists, assuming that all questionnaires were distributed (Fig. 3.1.1). The rates of return per region based on 100 questionnaires sent to each region were as follows: Central 64%; Eastern 49%; Interlake 48%; Southwest 24%; and Northwest 13%. Two questionnaires were anonymous and could not be assigned to a region. Of those who responded 64 (32% of respondents) were from the Central; 49 (25% of respondents) from Eastern; 48 (24% of respondents) from Interlake; 24 (12% of respondents) from Southwest; and 13 (7% of respondents) from Northwest region. Seventy-two per cent of the respondents were beef producers while 22% were dairy and six per cent were mixed producers (both beef and dairy) (Fig. 3.1.2).

More beef producers responded in each region followed by dairy producers. Most of the dairy producers who responded were from the Central and Eastern regions (representing 59% and 27% of dairy producers who responded, respectively) (Fig. 3.1.1).

A total of 162 (81%) producers indicated that they were tagging their cattle to control flies, while 38 (19%) were either using other control methods or not protecting their cattle at all (Fig. 3.1.3). The proportion of tag users among the respondents was similar in all regions. More than 80% of the respondents in each region were using or have used tags, with the exception of the Interlake region where 71% of the respondents were using tags. Fifty-four per cent of those who were tagging have used ear tags for four to six years; 44% have used ear tags for one to three years, while less than 2% have used ear tags for more than six years, most of the latter were beef producers from the central region. This last category of producers started using tags before they were registered in Canada, and might have bought them in the U.S. Interestingly, these producers were applying less than one tag per animal and claimed to be achieving satisfactory control.

Most producers (59% of respondents using tags) were tagging at the recommended rate of one tag per animal for horn fly control in Manitoba (Anonymous 1987, 1989) (Fig. 3.1.3). Thirty-seven per cent were tagging below the

recommended rate and a small proportion (2% of respondents using tags) were applying two tags per animal, the rate recommended for reduction of face flies (Anonymous 1987, 1989). Producers in this last category were from the Eastern region. About two per cent were using more than one tagging rate for certain groups of animals, for example, two tags for bulls and one tag for cows. The proportion of producers applying less than one tag per animal and one tag per animal was similar among beef producers, 43% and 54%, respectively. Among dairy producers, a majority (71%) were applying one tag per animal compared to 19% applying less than one tag per animal.

Half of the producers tagging at less than one tag per animal responded that ear tags were offering fair control while 45% responded that ear tags were very good in controlling flies (Fig. 3.1.3). Half (50%) of those applying one tag per animal were achieving very good control of flies while 48% responded that ear tags were offering fair control of flies. At both tagging rates, less than 5% indicated that tags were affording poor control. However, these results must be treated with caution as some producers cannot differentiate horn flies from other flies. Some farms where producers had indicated poor control were visited with the aim of collecting horn flies, only to find house flies, mainly in barns.

Most of the respondents using ear tags were beef producers (75%), while dairy and mixed producers represented 19% and 6%, respectively (Fig. 3.1.2). About 85% of beef producers and 70% of dairy producers were using tags. Only 19% of respondents (38 producers) were not using tags (Fig. 3.1.3). Of these, 41% were spraying their cattle while another quarter (25.5%) were not using any control measures. Eighty per cent of producers who were not using any control measures against flies were beef producers. Eighty-eight per cent of producers using sprays were dairy producers who were mainly spraying cattle when they were brought into the barn for milking using ready-to-use aerosol spray formulations. Thirty-four per cent of the producers not using ear tags were using oilers while 20% were using backrubbers. Among the producers using oilers, the proportion of dairy and beef producers was similar (48%). None of the producers were using dust bags, which may be attributed either to poor availability of insecticide formulations for use in dust bags or to lack of recommendations for insecticides for use in dust bags (Anonymous 1987, 1989) (Fig. 3.1.3).

A sizeable majority (89%) of respondents using ear tags have used fenvalerate-impregnated ear tags while 38% have used permethrin-impregnated ear tags. Some producers have used one tag type only. Sixty per cent of tag users have used fenvalerate tags only while 9% have used permethrin

tags only. The remaining 31% of tag users reported that they have used more than one brand of tags. Of these, 26% have used both fenvalerate and permethrin ear tags. In addition to tags mentioned above, some producers have used tetrachlorvinphos- and cypermethrin-impregnated ear tags (8% and 3% of tag users, respectively). It was not possible to tell from the questionnaire either the order in which the ear tags were used or the reasons for the changing to other brands. Among the pyrethroids used in ear tags, fenvalerate was the first to be registered and this might explain why more producers have used fenvalerate tags. Permethrin and cypermethrin tags were registered in 1984 and 1986, respectively (S. Van Walleggem, pers. comm.).

Clearly, ear tags have been rapidly accepted by the majority of producers in Manitoba and have gained widespread use. Ear tags were registered for fly control on cattle in 1981 in Canada, and 56% of producers in Manitoba using tags (45% of total respondents) have been using tags since 1983 while the remainder (35% of total respondents) started using tags after 1983. Both beef and dairy producers seem to have accepted ear tags over other previously available methods. Ear tags were accepted earlier by beef producers than dairy producers. Sixty per cent of beef producers who use tags started using them before 1984 while the remaining 40% started using tags after 1983. The reverse is true for dairy producers; 61% of dairy producers using ear tags began

using them after 1983.

In the United States, horn fly resistance to insecticides in ear tags developed within four years of their registration for use (Kunz and Schmidt 1985a; Quisenberry *et al.* 1984; Roush *et al.* 1986; Schmidt *et al.* 1985; Sheppard 1984). In Manitoba, a considerable number of producers (56% of tag users) have used ear tags for more than four years and have indicated that they are still achieving satisfactory control with ear tags. Differences in the rate of resistance development between horn fly populations in the U.S. and Manitoba could be attributed to differences in the selection pressure being exerted on these populations. The horn fly season in the U.S. may last for more than 5 months (Kunz and Cunningham 1977; Thomas and Kunz 1986; Thomas *et al.* 1987) compared to approximately 3 months in Manitoba and Canada (Depner 1961; McLintock and Depner 1954). In addition, the summer generation time of horn flies in the southern U.S., where resistance appeared first, is shorter (9 to 10 days) (Kunz *et al.* 1970) than that of horn flies in Manitoba (more than 14 days) (Depner 1961; McLintock and Depner 1954). Selection pressure for resistance is exerted on many generations over a longer time in the U.S. compared to Manitoba. Over 95% of producers using ear tags at less than one tag per animal or at one tag per animal, were satisfied with the control being afforded by the ear tags. Thus, up to spring of 1987 when the survey

was conducted, it appears most horn fly populations in Manitoba could still be controlled with ear tags and could be regarded to be susceptible to pyrethroids.

On the basis of these results, it seemed important to carry out an extensive field survey of horn fly populations in Manitoba to evaluate their susceptibility or tolerance to pyrethroids. Where very few flies are collected, fenvalerate must be evaluated first because it is used widely. If indeed, horn fly populations in Manitoba have low levels of resistance to pyrethroids in ear tags, despite their use for the same period as in the U.S.A., an effort must be made to establish the responsible factors. Such factors must be capitalized on to delay the onset of higher levels of resistance to pyrethroids in ear tags so that producers can enjoy the ease of application and effectiveness of ear tags for a longer period.

Figure 3.1.1. Return rate of questionnaires distributed to cattle producers in Manitoba with total numbers received from each region (% of total in each category).

Total
Distributed

No. Returned

Region

Enterprise

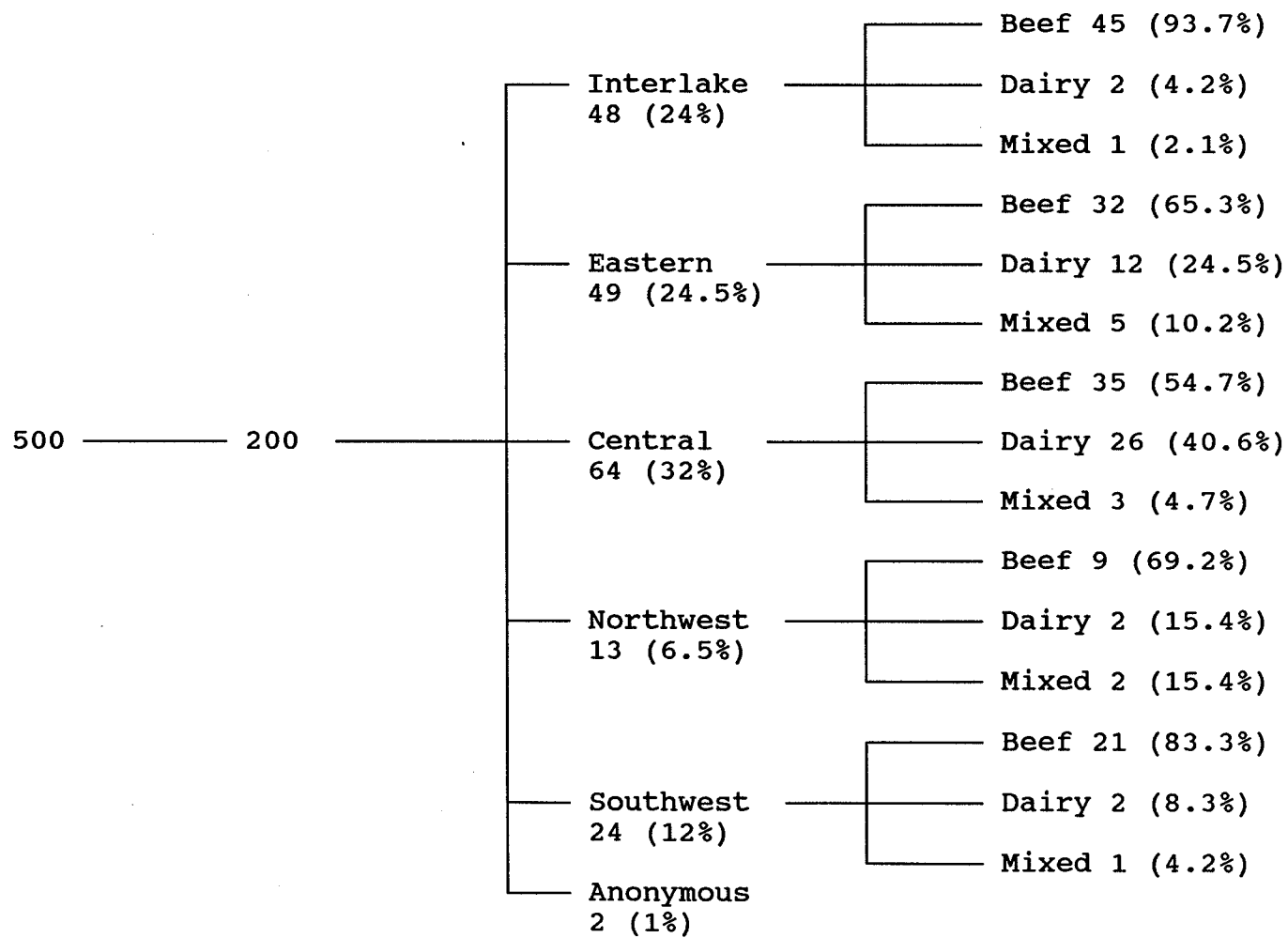


Figure 3.1.2. Proportion of responding Manitoba cattle producers applying tags within each enterprise.

Total Response

200

Enterprise

Beef
144 (72%)

Dairy
44 (22%)

Mixed
12 (6%)

Proportion
Tagging

Tagging
122 (85%)

Not tagging
22 (15%)

Tagging
31 (70%)

Not tagging
13 (30%)

Tagging
9 (75%)

Not tagging
3 (25%)

Figure 3.1.3. Length of insecticidal ear tag use, tagging rates and control acheived by producers in Manitoba. Numbers are total questionnaires received in each category and % in each category.

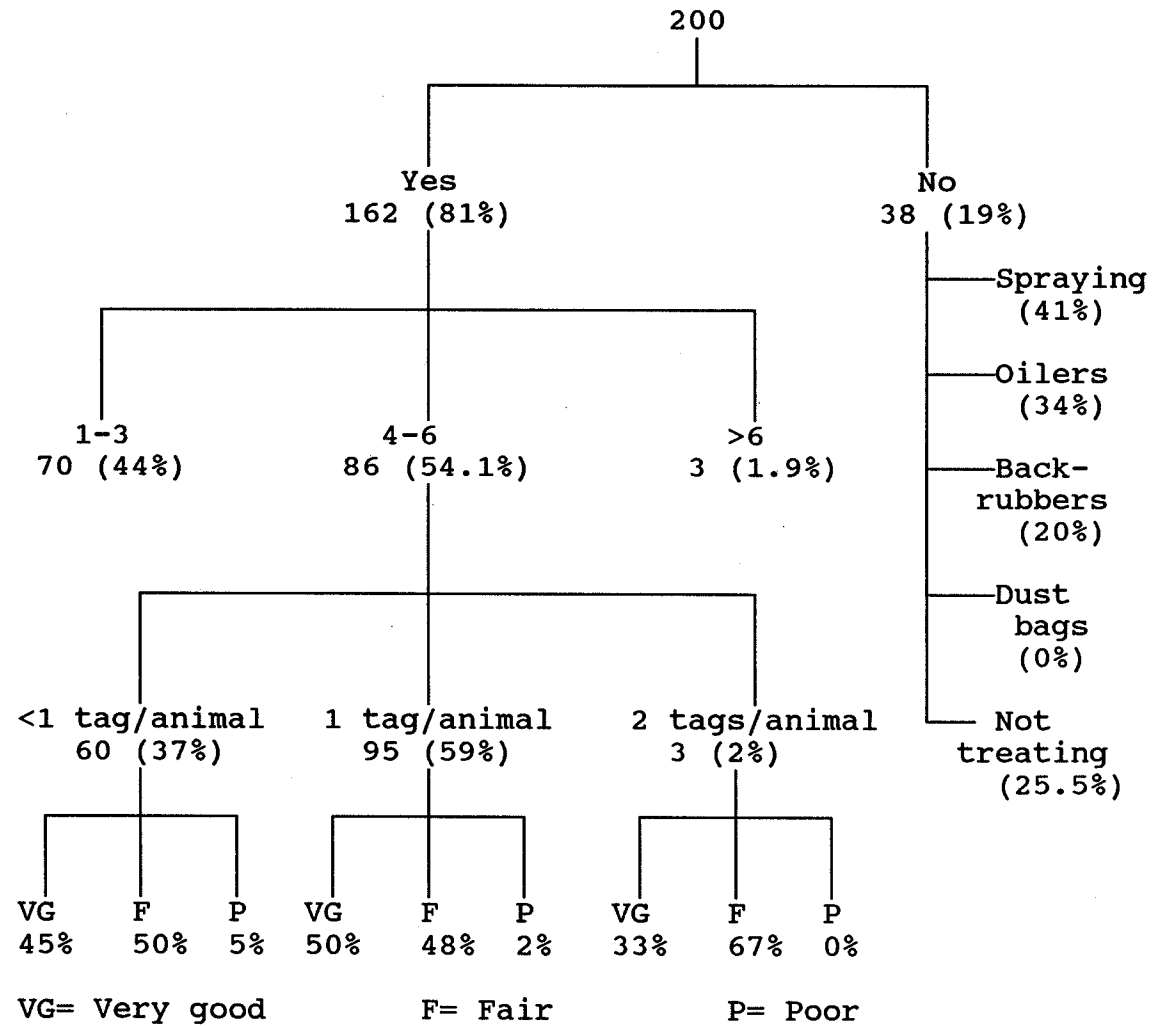
Total response

Tagging

Years of tag use

Tagging rate

Control achieved



CHAPTER III

SECTION II

SUSCEPTIBILITY OF HORN FLIES, *HAEMATOBIA IRRITANS* (L.), TO PYRETHROIDS IN MANITOBA.

Abstract

Horn fly, *Haematobia irritans* (L.), resistance to fenvalerate and permethrin was evaluated in Manitoba from 1987 to 1989. The number of resistant populations and intensity of resistance increased during the period. In 1987, 18 herds were sampled from the Eastern, Central and Interlake regions. Tolerant flies were observed on 3 herds and resistance factors at LC_{50} were not more than 6-fold for fenvalerate and 3-fold for permethrin compared to the susceptible strain except for flies from a herd near Carman that were 14-fold resistant to permethrin. In 1988, 26 herds were sampled from the above and Southwest regions. Six resistant populations were found and resistance factors ranged from 0.03- to 38-fold for fenvalerate and 0.1- to over 100-fold for permethrin. In 1989, 22 herds were sampled from the Eastern, Central and Interlake regions. Resistant flies were found on 10 herds and resistance factors ranged from 1.0- to 62-fold for fenvalerate and 0.8- to over 100-fold for permethrin. In 1988, only one resistant population was found near the border with the U.S. but in 1989 two other highly resistant populations were found in the southern Eastern region, one of which had no flies in 1988. It is hypothesized that these resistant flies dispersed from the U.S. into Manitoba, rather than developing resistance independently.

Introduction

Pyrethroid resistance in horn flies, *Haematobia irritans* (L.), is a major concern to cattle producers and veterinary entomologists. Horn fly resistance to pyrethroids became a problem with the introduction and widespread use of pyrethroid-impregnated ear tags (Kunz and Schimdt 1985a; Sparks et al. 1985). Insecticidal ear tag technology not only provided producers with an effective method of controlling horn flies for the whole season with a single treatment (Ahrens and Cocke 1979; Knapp and Herald 1981; Williams and Westby 1980), but also with an environmentally sound method that reduced the amount of insecticide applied by over 98% compared to conventional sprays (Kunz and Schmidt 1985a). However, because insecticidal ear tags continuously release insecticide over a prolonged period at a progressively decreasing rate (Miller et al. 1983a), the inevitable occurred; horn flies rapidly developed resistance to insecticides incorporated in ear tags. Resistant horn fly populations were reported after 2-4 years of continuous use of insecticidal ear tags in the United States (Kunz and Schimdt 1985a; Quisenberry et al. 1984; Schmidt et al. 1985; Sheppard 1984;).

In Canada, insecticidal ear tags were registered in 1981 (S. van Wallegghem, Ag. Canada, pers. comm.) but there were no confirmed cases of horn fly resistance to pyrethroids in ear tags before this study was initiated in

1987. In a producer survey conducted in spring of 1987 in Manitoba, over 95% of producers surveyed still achieved satisfactory horn fly control with insecticidal ear tags (Chapter III, Section I). The objective of this study was to determine the susceptibility of horn flies to pyrethroids in Manitoba, Canada.

Materials and Methods

Technical grade materials of fenvalerate and cypermethrin (Ciba Geigy, Canada); permethrin (*cis:trans*, 40:60) and λ -cyhalothrin (Coopers Agropharm, Inc. Canada) were used. Permethrin and fenvalerate were included in all bioassays while cypermethrin and λ -cyhalothrin were tested occasionally in 1988 and 1989, respectively.

Adult horn flies were collected from cattle at various locations in Manitoba in 1987 to 1989. Flies were transported in cages on ice to the laboratory and bioassayed within 4 hours of collection.

A susceptible horn fly strain was established from pupae obtained from USDA, Kerrville, Texas. Adult flies were maintained at 28 ± 1 °C under a 16:8 (L:D) photoperiod and 60% RH in screened cages (30 x 30 x 30 cm), and fed citrated bovine blood without antibiotics (Kunz and Schmidt 1985b). Eggs were collected and seeded in 250 mL plastic cups containing fresh manure from untreated steers. The cups were held for 7 days at 28 ± 1 °C and 60% RH. Pupae were

separated from the manure by floatation, dried and put in cages (30 x 30 x 30 cm) for adult emergence.

Flies were exposed to residues of pyrethroids on filter papers in petri-dishes according to the method of Sheppard and Hinkle (1987). Filter papers were impregnated with serial concentrations of technical grade insecticide in acetone ranging from 0.0195 $\mu\text{g}/\text{cm}^2$ to 20 $\mu\text{g}/\text{cm}^2$. In 1987 and 1988, concentrations ranging from 0.0195 to 10 $\mu\text{g}/\text{cm}^2$ were used. In 1989, two more concentrations of 15 and 20 $\mu\text{g}/\text{cm}^2$ were included in tests. Ten to fifty flies were tested per petri-dish and each concentration was replicated twice. When samples were too small to run a full bioassay, flies were exposed to discrimination concentrations of 0.625 $\mu\text{g}/\text{cm}^2$ of fenvalerate and 5 $\mu\text{g}/\text{cm}^2$ of permethrin. The discrimination concentrations chosen were the next concentrations in the bioassay range above concentrations that killed 100% of the susceptible laboratory flies. Two controls with filter paper treated with acetone only were run concurrently. Three to five day old flies from the susceptible laboratory colony were tested occasionally in 1987 and 1988. The colony died out in spring of 1989 and attempts to re-establish it failed; consequently, no tests with the susceptible laboratory strain were conducted in summer, 1989.

Mortality was assessed immediately after flies were introduced into petri-dishes and 2 hours later. Flies that

could not fly or walk were considered dead.

Mortality data were subjected to Probit analysis using POLO-PC (Le Ora Software, Inc. 1987). Concentrations that killed 50% of the flies (LC_{50} 's) were considered significantly different if their 95% confidence limits (CL) did not overlap. Resistance factors were calculated by dividing the LC_{50} of each field population by the LC_{50} of the susceptible strain. Data for bioassays conducted in 1987 and 1988 on the susceptible strain were pooled to generate one regression line. Correlation analysis (SAS Institute, Inc. 1985) was used to test for association between pairs of LC_{50} 's for fenvalerate and permethrin.

Results

From 1987 to 1989, 49 herds were sampled for horn flies. Eighteen herds were sampled in 1987. Horn flies were found on 8 herds; five of which had tags (Table 3.2.1, 3.2.2, 3.2.3). Resistance factors for flies from these herds ranged from 0.1- to 5.5-fold for fenvalerate and 0.58- to 14-fold for permethrin. With the exception of flies from herd 8 near Carman, resistance factors to fenvalerate were less than 6-fold and less than 3-fold for permethrin. Flies tolerant to fenvalerate and permethrin were collected from three herds (8, 10 and 18) (Tables 3.2.1, 3.2.2).

In 1988, horn flies were collected from 10 herds, out of 26 herds that were sampled (Tables 3.2.1, 3.2.2, and

3.2.3). Resistance factors relative to the susceptible strain ranged from 0.03- to 38.44-fold for fenvalerate and 0.11- to >>100-fold for permethrin. Resistant flies were collected from six herds, most of which were more than 8-fold resistant to fenvalerate. No flies were observed on herd 10 near Morden which had permethrin tags. A small sample of flies that was collected in 1987 when the herd was tagged with fenvalerate tags had resistance factors of 5.50-fold for fenvalerate and 1.94-fold for permethrin at discrimination concentrations. Flies collected from bulls near Pipestone (herd 38), were 25.67-fold and 12-fold resistant to fenvalerate and permethrin, respectively. Two new fenvalerate tags were applied on the bulls but fly numbers were not appreciably reduced. Flies sent from Estevan, southeastern Saskatchewan, were 28.33-fold more resistant to fenvalerate than the susceptible strain. For permethrin, mortality was observed at the highest concentration used in the bioassay range (12.5% mortality at $10 \mu\text{g}/\text{cm}^2$).

Fly samples that were exposed to cypermethrin had LC_{50} 's ranging from approximately 2 to $5 \mu\text{g}/\text{cm}^2$ (Table 3.2.4). Resistance factors could not be calculated for this insecticide because it was not assessed against the susceptible laboratory strain. Overall, cypermethrin was 0.9 to 2.5 times more toxic to the flies than fenvalerate and 2 to 8.5 times more toxic than permethrin.

In 1989, 22 herds were sampled and flies were collected from 15 herds. Resistance factors for fenvalerate ranged from 1.0- to 61.5-fold (Table 3.2.1 and 3.2.2). Flies from 10 herds (herd 18, 20, 24, 25, 26, 40, 41, 44, 45 and 46) were more than 10-fold resistant to fenvalerate; flies from the other 5 herds (8, 17, 23, 42 and 43) were less than 3-fold tolerant to fenvalerate (Table 3.2.1 and 3.2.2). Resistance factors for permethrin ranged from 0.76- to >>100-fold. For flies from herds 26, 44 and 46, mortality was observed at the highest concentration of permethrin used in the bioassays. Mortality at 20 $\mu\text{g}/\text{cm}^2$ of permethrin was 29% for flies from herd 26 and 74% for flies from herd 46 and 17% at 15 $\mu\text{g}/\text{cm}^2$ for flies from herd 44. Resistance factors in seven horn fly populations (herds 18, 20, 24, 25, 40, 41 and 45) were above 10-fold for permethrin, whereas resistance factors from herds 8, 17, 23, 42, and 43 were less than 3-fold.

The LC_{50} 's for flies from herds 18 and 25 were approximately 0.28 and 0.35 $\mu\text{g}/\text{cm}^2$ of λ -cyhalothrin, respectively. λ -cyhalothrin was 7 to 11 times more toxic to these flies than fenvalerate and over 39 times more toxic than permethrin.

Though the response of horn fly populations to the pyrethroids at LC_{50} was not consistent, the order of toxicity to the tested field flies was λ -cyhalothrin > cypermethrin $\geq$ fenvalerate > permethrin. On average,

fenvalerate was about 5 times more toxic to the flies than permethrin. The LC_{50} 's of the flies to fenvalerate and permethrin were positively correlated ($R^2=0.67$, $P=0.0004$). Flies with resistance factors ranging from 0.4- to 2.3-fold were not significantly different from the susceptible laboratory strain (overlap of 95% CL). Flies from untreated herd 2 (Ashern) were more susceptible to fenvalerate and permethrin than the laboratory strain in both 1987 and 1988 while flies from another untreated herd 23 (Portage la Prairie) were significantly more susceptible to both insecticides than the laboratory strain in 1988 but not in 1989.

Discussion

Significant variations in the susceptibility of horn fly populations in Manitoba were observed. The number of resistant horn fly populations and intensity of resistance increased during the study period. Resistant populations were widely interspersed among susceptible populations. Although some populations collected from untreated herds were more susceptible than the Kerrville susceptible strain, the response of most populations was not significantly different from the Kerrville strain (overlap of 95% CL). The Kerrville strain is, therefore, still a good representative of susceptible populations despite being colonized in the laboratory for over 16 yr.

The horn fly population at Glenlea Research Station was the first case of resistance encountered in Manitoba. This population was 5.2-fold resistant to fenvalerate and 2.5-fold to permethrin in 1987, and there were slightly fewer flies ($P>0.05$) on cattle treated with two permethrin tags than those that had two fenvalerate tags (Chapter III, Section IV). Horn flies were also not observed on herd 10 in 1988 and 1989 when permethrin tags were applied at one tag per animal. This population was 5.5-fold resistant to fenvalerate and 2-fold to permethrin in 1987. Horn fly populations that are less than 3-fold tolerant to pyrethroids can be considered susceptible. Kunz and Schmidt (1985a) stated that resistance to pyrethroids can be considered to cause failure of ear tag treatment when it approaches 3-fold. They based their observations on data established with the insecticide-impregnated cloth method. The data obtained with the impregnated filter paper method support their observations. Flies with resistance factors below 3-fold may be indicative of vigour tolerance (Hoskins and Gordon 1956). But when the resistance factors approach 3-fold, continued application of ear tags may rapidly lead to control failure (Chapter III, Section IV).

Cross-resistance patterns observed among the pyrethroids evaluated were variable. For example, among the resistant fly populations that were evaluated in 1988 against fenvalerate, permethrin and cypermethrin, flies from

Libau were the most resistant to fenvalerate and cypermethrin but least tolerant to permethrin. Flies from Glenlea and Pipestone were the least tolerant to fenvalerate and cypermethrin, respectively. On average, there was a positive correlation between the response of the flies to fenvalerate and permethrin. Flies that were resistant to fenvalerate were also resistant to permethrin, and fenvalerate was as toxic to the flies as cypermethrin. Byford et al. (1985), using a topical application method, found that fenvalerate was as toxic to their susceptible strain as cypermethrin, but the resistant populations were more resistant to cypermethrin than fenvalerate. Based on the LC_{50} 's observed for fenvalerate and cypermethrin in 1988, the response of the horn fly populations to both compounds was similar. Resistance to λ -cyhalothrin appears to be negatively correlated with fenvalerate and permethrin in that flies that were more resistant to fenvalerate and permethrin had lower LC_{50} 's to λ -cyhalothrin, while flies that were less resistant had higher LC_{50} 's. However, the number of fly populations assessed was too small for firm conclusions to be drawn, but the level of cross-resistance to λ -cyhalothrin in these populations appears to be low. Tags containing λ -cyhalothrin controlled a fly population that was 15-fold resistant to fenvalerate and 12-fold resistant to permethrin in field trials (Chapter III, Section VI). Sparks and Byford (1988) and Leonard et al.

(1988) found very low levels of cross-resistance to λ -cyhalothrin in horn flies and tobacco budworm (*Heliothis virescens* (F.)), respectively.

In 1987, resistance factors for fenvalerate and permethrin were less than 6-fold and 3-fold, respectively except for flies from herd 8. Most of the herds sampled were tagged and did not have flies on them (Map 3.2.1). This is in agreement with a survey on tag usage and horn fly control conducted in spring of 1987, when most producers were still achieving at least fair horn fly control with ear tags (Chapter III, Section I). In 1988, resistant populations were found in the Eastern, Southwest and Interlake regions but not in the Central region (Maps 3.2.2 and 3.2.3). Only one resistant fly population (herd 25) was found south of Steinbach in 1988. In 1989, more highly resistant populations were found south of Steinbach (Maps 3.2.4 and 3.2.5). Herd 26 had no flies in 1988. In 1989, a highly resistant population was found on this herd. Another herd in the same region (herd 4) had no flies in 1987 and 1988. In 1989, large numbers of flies were observed on tagged cattle in herd 4 but flies could not be sampled for bioassay. The above evidence, coupled with the fact that lower resistance levels were observed at Glenlea Research Station over two years of selection (Chapter III, Section IV) than those observed in the southern eastern region in 1989, support the hypothesis that resistant flies might be

dispersing from the south into Manitoba. It is implausible that such levels of resistance could be attained in a year.

Assuming similar initial gene frequencies in horn fly populations in Manitoba and southern U.S., horn fly populations in Manitoba are expected to develop resistance more slowly than populations in southern U.S.. This is so because the fly season is longer in southern U.S. where the season may be 8 to 12 months (Hogsette and Ruff 1986; Kunz and Miller 1985; Thomas and Kunz 1986) than in most parts of Canada, where the season is less than 6 months (Depner 1961). Selection pressure is therefore exerted on more generations in a season in southern U.S. than in Manitoba, and resistance is expected to develop slowly in the latter (Georghiou 1980; Georghiou and Taylor 1977a, b; Tabashnik and Croft 1982). Another factor that might have contributed to rapid development of resistance in horn fly populations in the southern U.S. is the concentration gradient of insecticide created by ear tags (Chapter III, Section III; Miller et al. 1983a, 1986; Taylor et al. 1985). Ear tags applied to cattle in southern U.S. would be releasing much lower residues at the end of the season than experienced at the end of the season in Manitoba. Lower residues may select for resistance rapidly because they allow flies heterozygous for resistance to survive (McDonald et al. 1987a; Roush et al. 1986; Sparks et al. 1985).

There is considerable evidence in the literature to support the contention that horn flies can disperse over long distances. Although Chamberlain (1981, 1982) and McLintock and Depner (1954) noted that horn flies do not disperse beyond the boundaries of a herd, many authors have demonstrated that horn flies can disperse long distances in short periods of time (Beadles *et al.* 1975; Byford *et al.* 1987a; Eddy *et al.* 1962; Kinzer and Reeves 1974; Kunz *et al.* 1983; Skoda *et al.* 1987; Tugwell *et al.* 1966). Marked flies have been recovered at a distance of 26 km from release sites within 14 days (Beadles *et al.* 1975) and 12 km in 10 hr (Kinzer and Reeves 1974). These extended flights are made by newly eclosed horn flies searching for their first host (Chamberlain 1982; Guillot *et al.* 1988; Tugwell *et al.* 1966). Further evidence of horn fly capability to disperse can be obtained from records on the spread of horn flies following their introduction into North America. Horn flies infested most cattle herds within 8 yr following their first record in the U.S. (Hoelscher *et al.* 1968) and 6 yr in Canada (McLintock and Depner 1954). McDonald *et al.* (1987b) assessed the genetic variability of horn fly populations in North America. They found few genetic differences among the populations and concluded that there were no barriers to gene flow among the populations. Furthermore, horn flies from California, Florida, and Texas had similar resistance genes to pyrethroids (McDonald and Schmidt 1990). Horn fly

populations in North America are therefore not genetically isolated and may be interbreeding freely.

The pattern in which horn fly resistance developed and spread in North America supports a hypothesis of dispersal of resistant horn flies. Resistant horn fly populations did not appear to arise discretely, but spread through U.S. in a northwesterly fashion (Kunz and Schmidt 1985a). Pyrethroid-resistant horn flies were first reported in Florida in 1982 (Kunz and Schmidt 1985a). At the end of 1984, resistant horn flies were confirmed in Louisiana, Oklahoma, Kansas, Kentucky, Georgia, Texas, Nebraska, California and Hawaii, and suspected in Mississippi, Alabama, Tennessee and Arkansas (Kunz and Schmidt 1985a). By 1987, resistant populations were reported in 24 states in the southeastern and mid western U.S. as well as Hawaii, California and Oregon (Marchiondo 1987) (Map 3.2.6). The most northern state in which resistant populations were reported was North Dakota (Marchiondo 1987; Meyer and Kopp 1986) which shares a border with Manitoba and eastern Saskatchewan. In this survey, resistance to pyrethroids in horn flies in Manitoba and southeastern Saskatchewan was confirmed. As of summer 1989, there was no evidence of horn fly resistance to pyrethroids in Ontario (G.A. Surgeoner, Univ. of Guelph, pers. comm.) and Alberta (D. Colwell, Lethbridge Research Station, pers. comm.). In 1989, resistance was suspected in central British Columbia (H.G. Philip, British Columbia

Agriculture, pers. comm.). Pyrethroid ear tags have been used for the same period in these provinces. It is strongly suspected that resistant flies are dispersing from the south on southerly winds. Chamberlain (1985) trapped more horn flies downwind from the release site and at greater heights as the distance from the release site increased. It is probable that horn flies utilize wind to assist dispersal. Wind-borne dispersal was implicated in the spread of the closely related buffalo fly, *Haematobia irritans exigua* (de Meijere), from the Australia mainland to Mornington Island (Tillyard 1931) and Magnetic Island (Ferror 1969). Use of southerly winds by other insect species dispersing into Manitoba has been documented by Smith and MacKay (1989) in the pea aphid (*Acyrtosiphon pisum* (Harris)) and by Sellers and Maarouf (1988) in the mosquito (*Culex tarsalis* Coquillett). Dispersing resistant flies become established on treated herds rapidly, given the high reproductive capacity of horn flies (Kunz and Cumingham 1977). If dispersing flies are highly resistant to pyrethroids, they can survive residues deposited by fresh ear tags (McDonald et al. 1987a; Roush et al. 1986). Two new fenvalerate tags applied on bulls near Pipestone failed to control flies that were 25-fold resistant to fenvalerate. Another possible mode of horn fly dispersal is with transported cattle. However, resistance would have spread throughout the provinces of Canada if this was a major mechanism of

dispersal.

Dispersal of flies into a treated population can have an important influence on the development and management of resistance. Immigration of susceptible flies into a treated herd can delay the development of resistance by diluting the resistance genes (Comins 1977; Georghiou and Taylor 1977b; Tabashnik and Croft 1982). In contrast, if the immigrants are resistant, then resistance will develop rapidly in the population (Comins 1977). Once resistance has developed, it can be managed by using multiple insecticides either in a mosaic or alternating pattern (Byford et al. 1987c; Curtis 1981; Georghiou 1980, 1983).

The presence of pyrethroid resistance in horn fly populations in Manitoba is documented in this study. Populations exhibited varying levels of cross resistance to pyrethroids. Circumstantial evidence indicates that resistant flies may be dispersing from the south. Further studies are required to verify this hypothesis. If the hypothesis is correct, then resistance management programs must be implemented throughout North America and not regionally to be successful. Alternation of insecticides with different modes of action within the season may be the most expedient resistance management strategy.

Table 3.2.1. Response of horn flies collected from various locations in Manitoba to fenvalerate and permethrin, 1987-1989.

Herd no.	Location	Fenvalerate			Permethrin		
		LC ₅₀ (95% CL) ^a	Slope±S.E.	RF ^b	LC ₅₀ (95% CL)	Slope±S.E.	RF
	Susceptible	0.18(0.14-0.24)	3.57±0.16	1.00	1.31(1.07-1.61)	4.20±0.28	1.00
			1987				
1	Morden (98°06';49°11')	0.12(0.07-0.18)	3.75±0.48	0.67	1.47(1.19-1.74)	3.92±0.60	1.12
2	Ashern (98°02';51°11')	0.02(0.008-0.039)	1.23±0.23	0.11	0.76(0.57-0.92)	3.90±0.50	0.58
13	Grunthal (96°51';49°24')	0.16(0.12-0.21)	3.68±0.43	0.88	1.01(0.60-1.32)	5.78±1.10	0.84
18	Glenlea (97°08';49°38')	0.94 ^c	1.64±0.02	5.22	3.35(2.43-4.87)	2.01±0.51	2.48
			1988				
2	Ashern (98°02';51°11')	0.062(0.05-0.08)	2.46±0.29	0.34	0.23 ^c	1.41±0.45	0.18
18	Glenlea (97°08';49°38')	3.14(1.18-5.97)	2.98±0.29	17.44	15.58(10.79-47.69)	2.74±0.57	11.89
19	Teulon (97°16';50°23')	0.038(0.032-0.043)	4.89±1.41	0.21	0.86(0.46-1.20)	3.91±0.46	0.66
23	Portage (98°18';49°59')	0.005(0.00005-0.015)	1.29±0.77	0.03	0.15(0.03-0.22)	2.45±0.52	0.11
24	Libau (96°43'50°16')	6.92(5.63-9.06)	2.22±0.33	38.44	11.24 ^c	5.28±2.40	8.58
25	Vita (96°34';49°08')	2.16(0.79-3.99)	1.59±0.31	12.00	>>100	0.21±0.62	-
38	Pipestone (100°57';49°08')	4.62(3.96-5.32)	3.85±0.45	25.67	15.85(11.93-51.51)	3.32±1.23	12.10
39	Broomhill (100°13';56°58')	4.05(3.50-4.70)	3.13±0.33	22.50	38.0 ^c	1.65±1.16	29.00

Table 3.2.1. continued

Herd no.	Location	Fenvalerate			Permethrin		
		LC ₅₀ (95% CL) ^a	Slope±S.E.	RF ^b	LC ₅₀ (95% CL)	Slope±S.E.	RF
1989							
17	Narcisse (97°32';50°41')	0.43 ^c	1.12±0.36	2.39	1.11 ^c	0.53±0.34	1.27
18	Glenlea (97°08';49°38')	2.49(1.77-3.53)	1.07±0.39	13.83	13.78(9.61-42.38)	1.20±1.14	10.52
20	Lundar (98°02';50°42')	3.39±(1.76-7.19)	3.12±0.44	18.83	13.88 ^c	1.52±0.54	10.59
23	Portage (98°02';50°42')	0.34(0.11-0.58)	2.28±0.24	1.89	1.65(0.79-2.91)	1.61±0.19	1.26
24	Libau (96°43';50°16')	3.38(2.39-4.44)	2.29±0.42	18.77	16.98(14.06-18.44)	12.51±3.43	12.96
25	Vita (96°34';49°08')	3.20(2.37-4.49)	1.98±0.23	17.78	57.89(24.78-518.8)	0.88±0.21	44.19
26	Steinbach (96°16';49°32')	11.06(9.15-14.05)	3.36±0.52	61.50	- ^d		
40	Lundar (98°02';50°42')	1.98(1.61±2.42)	2.55±0.31	11.00	16.00(14.58-17.89)	7.61±1.53	12.21
41	Lundar (98°02';50°42')	2.76(2.26-3.39)	4.22±0.72	15.33	16.06(12.44-24.18)	4.79±0.99	12.26
42	Arborg (97°13';50°54')	0.20(0.08-0.28)	3.62±0.81	1.11	0.99(0.71-1.27)	3.24±0.68	0.76
43	Gunton (97°16';50°19')	0.41(0.23-0.63)	2.02±0.37	2.27	1.00(0.58-1.51)	1.90±0.36	0.76
44	Portage (98°18';49°59')	5.65(4.38-7.74)	2.59±0.39	31.38	- ^e		
45	Sundown (96°16';49°06')	5.30(3.99-7.94)	1.64±0.27	29.44	60.79(27.50-451-8)	1.11±0.27	46.40
46	Sundown (96°16';49°06')	7.12(6.83-13.04)	2.26±0.29	48.94	- ^e		

^aLC₅₀(95% confidence limits) in µg/cm².

^bResistance factor = LC₅₀ of field flies ÷ LC₅₀ of susceptible strain.

^cData too heterogeneous to calculate 95% CL (Le Ora Software, Inc. 1987)

^dMortality observed at 20 µg/cm² only.

^eMortality observed at 15 µg/cm² only.

Table 3.2.2. Responses of field-collected horn flies in Manitoba to discrimination concentrations of fenvalerate ($0.625 \mu\text{g}/\text{cm}^2$) and permethrin ($5 \mu\text{g}/\text{cm}^2$).

Year	Herd	Location	Fenvalerate		Permethrin	
			% Mortality	RF ^a	% Mortality	RF
		Susceptible	100	1.00	100	1.00
1987	8	Carman ($98^{\circ}00';49^{\circ}32'$)	22.22	4.50	7.14	14.0
	10	Morden ($98^{\circ}06';49^{\circ}11'$)	18.18	5.50	51.43	1.94
	11	Winkler ($97^{\circ}56';49^{\circ}11'$)	78.84	1.27	100	1.00
	16	Fisher Branch ($97^{\circ}37';51^{\circ}05'$)	95.24	1.05	100	1.00
1988	20	Lundar ($98^{\circ}02';50^{\circ}42'$)	10.53	9.50	20.91	4.78
	30	Ashern ($98^{\circ}02';51^{\circ}11'$)	100	1.00	100	1.0
1989	8	Carman ($98^{\circ}00';49^{\circ}32'$)	45.96	2.17	33.64	2.97

^aMortality in susceptible strain divided by mortality in field strains.

Table 3.2.3. Approximate locations of herds where no horn flies were observed on treated cattle in Manitoba from 1987 - 1989.

1987		1988		1989	
Herd	Location	Herd	Location	Herd	Location
3	Oak Point (98°02';50°29')	4	Sundown (96°16';49°06')	9	Carman (98°00';49°30')
4	Sundown (96°16';49°06')	10	Morden (98°06';49°11')	10	Morden (98°06';49°11')
5	Sundown (96°16';49°06')	14	Manitou (98°32';49°15')	11	Winkler (97°56';49°11')
6	Steinbach (96°16';49°32')	21	Clarkleigh (98°02';50°36')	12	Winkler (97°56';49°11')
7	Steinbach (96°16';49°32')	22	Seven Sisters (96°01';50°07')	47	Arborg (97°13';50°54')
9	Carman (98°00';49°30')	26	Steinbach (96°16';49°32')	48	Arborg (97°13';50°54')
12	Winkler (97°56';49°11')	27	Steinbach (96°16';49°32')	49	Arborg (97°13';50°54')
14	Manitou (98°32';49°15')	28	Steinbach (96°16';49°32')		
15	Fisher Branch (97°37';51°05)	29	Zhoda (96°31';49°17')		
17	Narcisse (97°32';50°41')	31	Ste. Anne (96°30';49°35')		
		32	Fisher Branch (97°37';51°05)		
		33	Fisher Branch (97°37';51°05)		
		34	Fisher Branch (97°37';51°05)		
		35	Altona (97°33';49°06')		
		36	Altona (97°33';49°06')		
		37	Altona (97°33';49°06')		

Table 3.2.4. Response of horn fly populations in Manitoba to cypermethrin and λ -cyhalothrin.

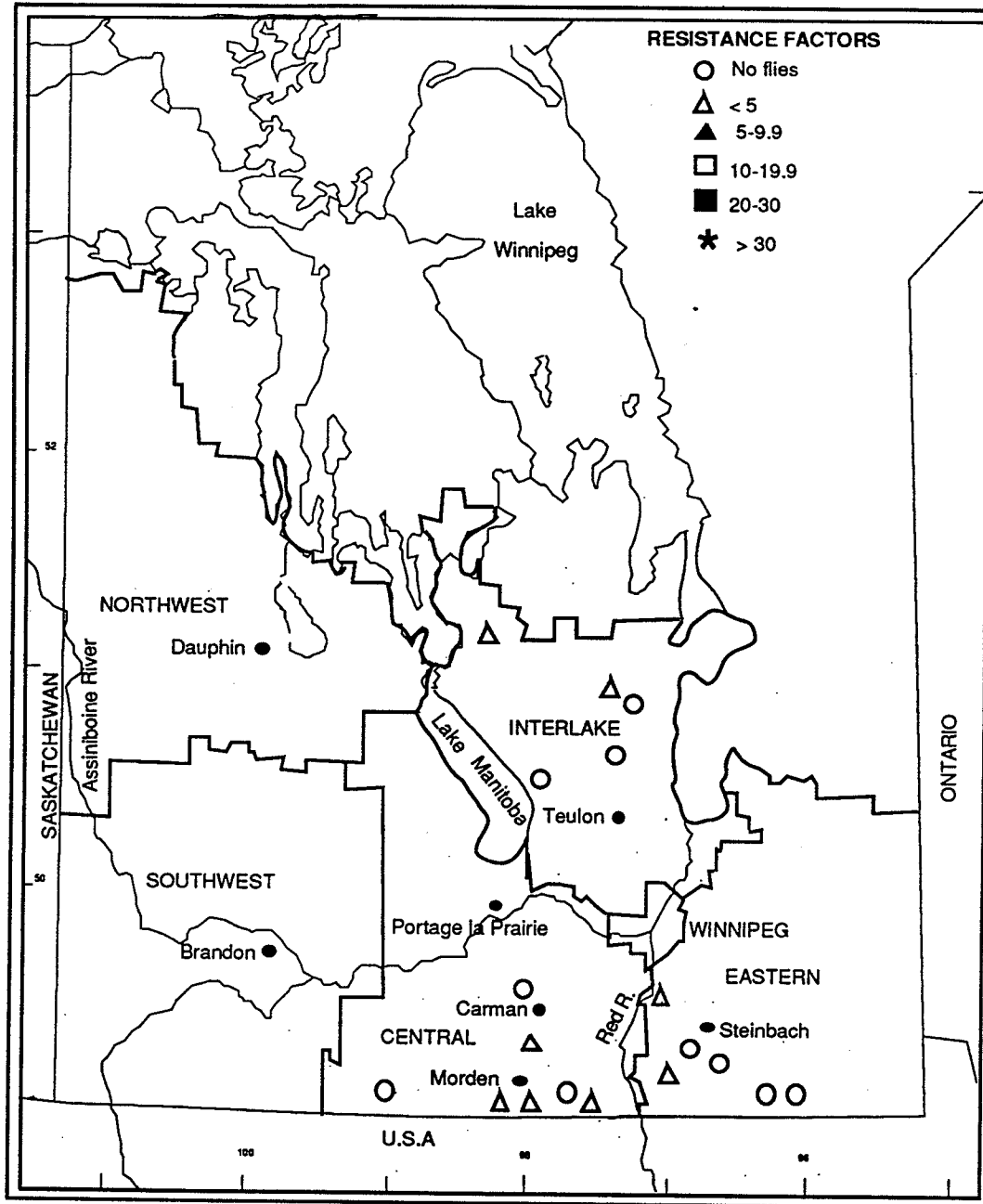
Year	Herd	Chemical	Location	LC ₅₀ ^a (95% CL)	Slope±S.E. ^b
1988	18	Cypermethrin	Glenlea	2.01 (1.70-2.48)	2.20±0.20
	24		Libau	4.98 (3.80-6.86)	2.86±0.33
	38		Pipestone	1.87 (0.75-3.56)	1.50±0.23
	39		Broomhill	4.34 ^c	1.77±0.55
1989	18	λ -cyhalothrin	Glenlea	0.35 (0.17-0.47)	1.17±0.46
	25		Vita	0.28 ^c	0.58±0.19

^aLC₅₀(95% confidence limits) in $\mu\text{g}/\text{cm}^2$.

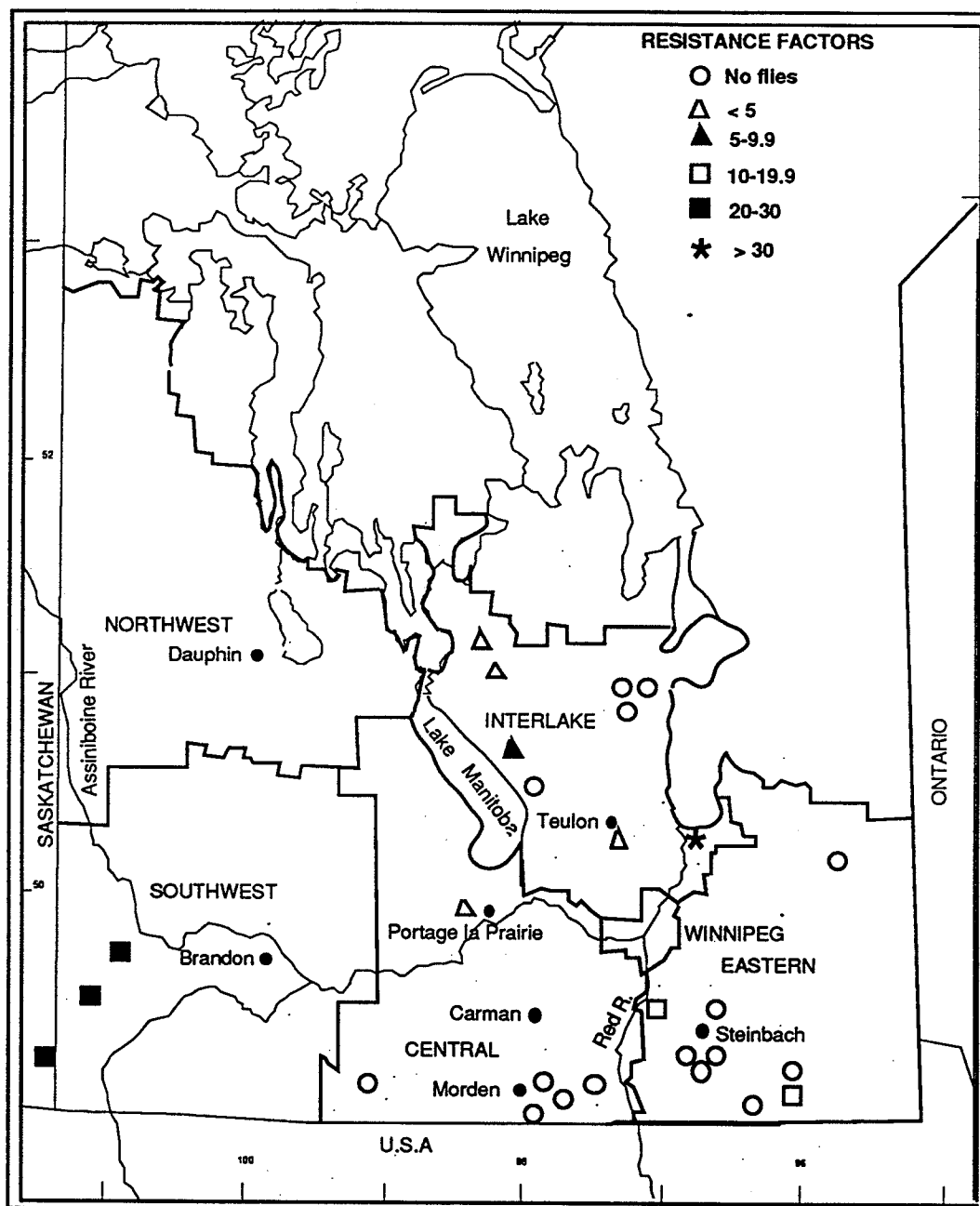
^bSlope and Standard error of log concentration mortality lines.

^cData too heterogeneous to calculate 95% CL; $g > 0.4$ (Le Ora Software Inc. 1987).

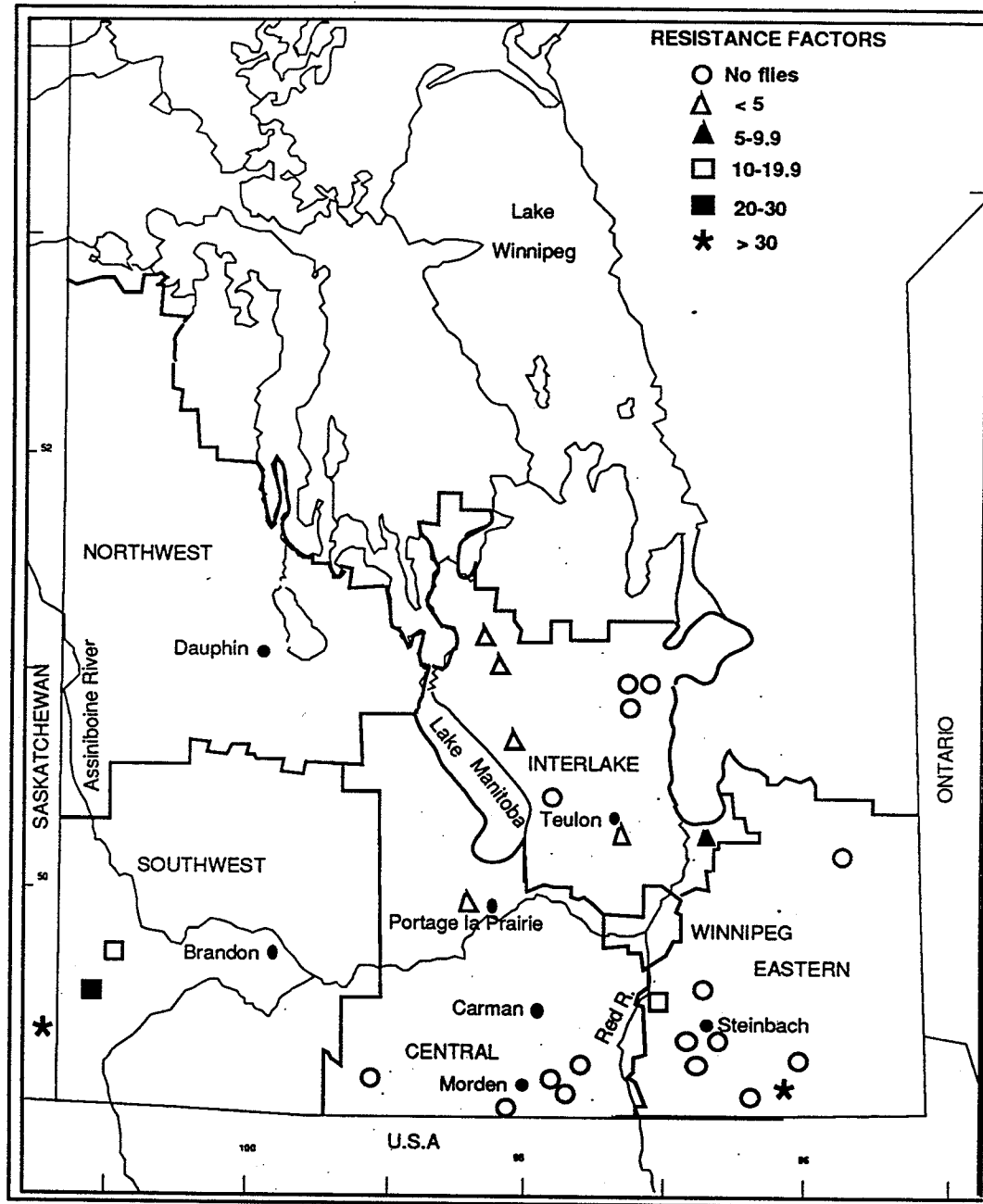
Map 3.2.1. Locations and resistance factors to fenvalerate and permethrin for horn fly populations sampled in Manitoba, 1987. Eighteen herds were sampled.



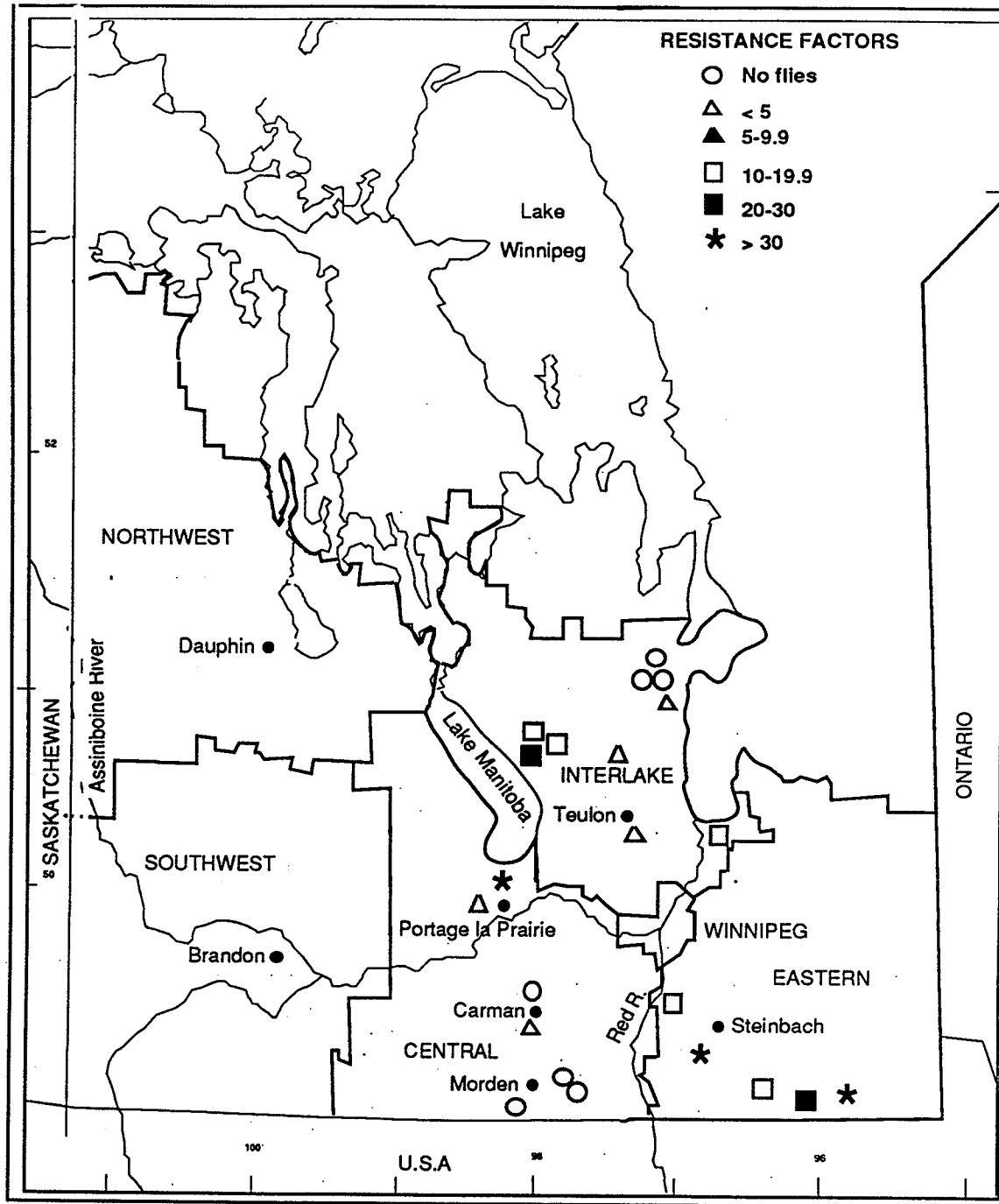
Map 3.2.2. Locations and resistance factors to fenvalerate
for horn fly populations sampled in Manitoba, 1988.
Twenty-six herds were sampled.



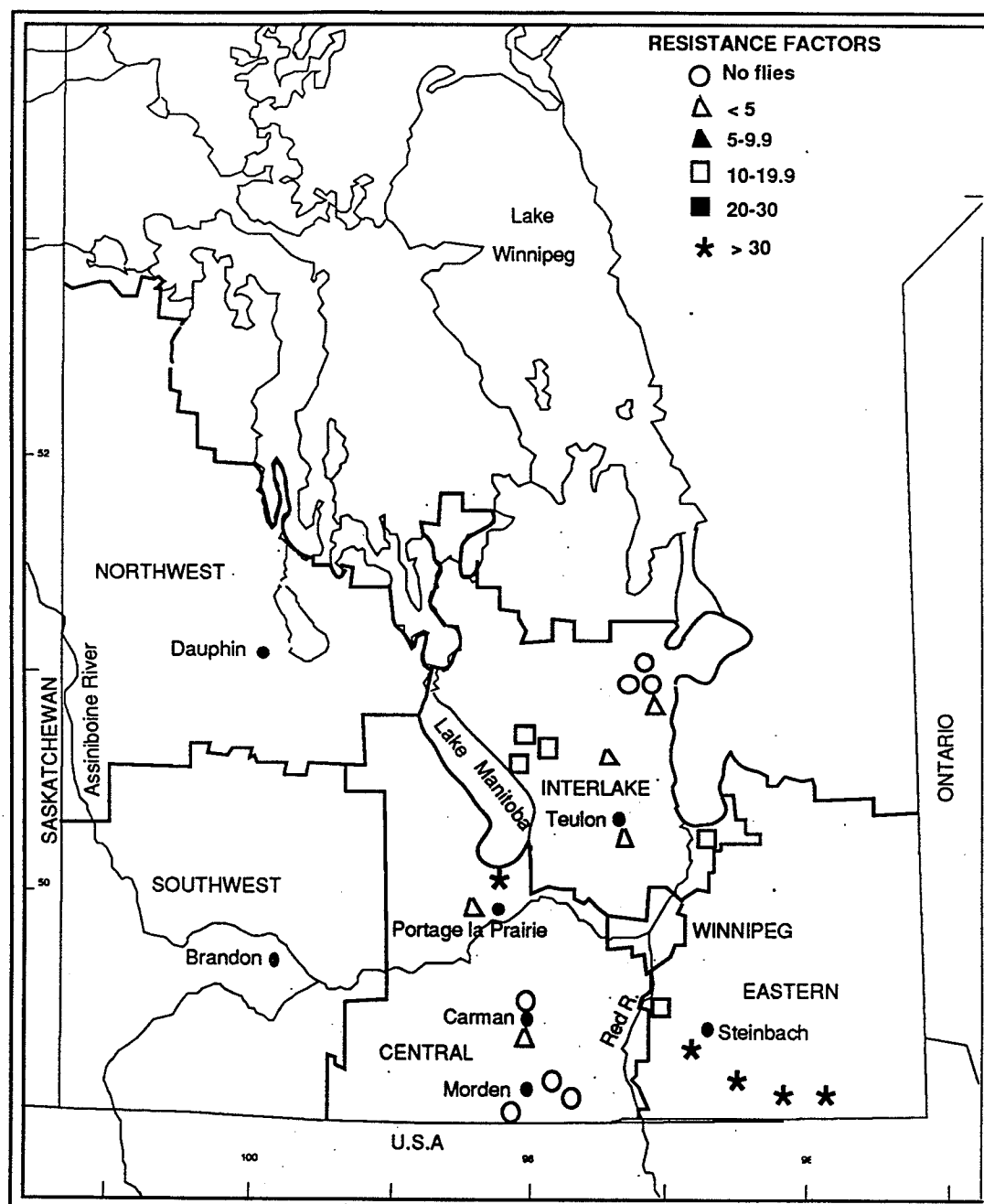
Map 3.2.3. Locations and resistance factors to permethrin
for horn fly populations sampled in Manitoba, 1988.
Twenty-six herds were sampled.



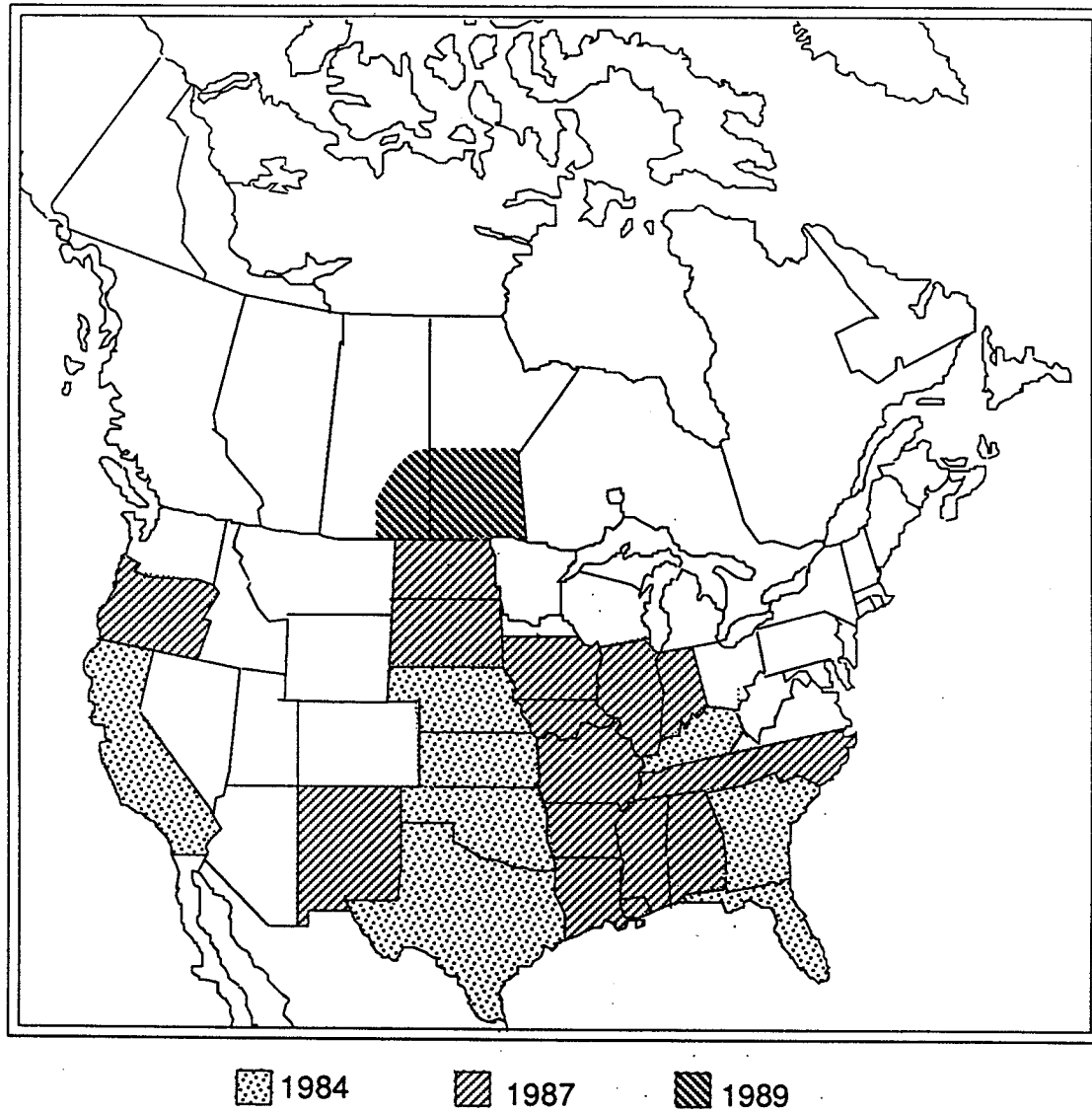
Map 3.2.4. Locations and resistance factors to fenvalerate
for horn fly populations sampled in Manitoba, 1989.
Twenty-two herds were sampled.



Map 3.2.5. Locations and resistance factors to permethrin
for horn fly populations sampled in Manitoba, 1989.
Twenty-two herds were sampled.



Map 3.2.6. Distribution and spread of horn fly resistance to pyrethroids in North America, 1981 to 1989.



Sources: Kunz and Schmidt, 1985; Marchiondo, 1987.

CHAPTER III**SECTION III**

**DISTRIBUTION OF PERMETHRIN AND FENVALERATE RESIDUES ON
CATTLE HAIR FOLLOWING VARIABLE APPLICATION RATES OF
IMPREGNATED EAR TAGS**

Abstract

Fenvalerate and permethrin residues on hair of cattle were determined over a period of 3 months following application of two tags per adult animal and one tag for every other adult animal in separately treated herds. Cattle with two tags had consistently higher residues than cattle with one tag. This difference was statistically significant ($P < 0.05$) in the first month for residues on the head and in the first 2 weeks for residues from the body. Residues on cattle with one tag and without a tag in the same herd were similar ($P > 0.05$) on each sampling day. Among the regions, residue levels on the hair from the head of cattle with two tags were greater than on the body and rump ($P < 0.05$) during the first 28 days. Residues on the hair from the body and rump regions of cattle with two tags were similar ($P > 0.05$) on each sampling day. There were no significant differences ($P > 0.05$) in residues found on all regions of cattle with and without a tag in the same herd on each sampling day. Residue levels on the head of cattle with two tags declined rapidly in the first month compared to those found on the body and rump. Residues found on hair on days 14 and 84 following tag application declined by 80-86% on the head, 73-78% on the body, and 36-86% on the rump. Isomeric compositions of fenvalerate (range 51-61% SR, RS:39-49% SS, RR) and permethrin (range 61-67% *trans*:33-39% *cis*)

were consistent during the study. Rainfall reduced residues on hair.

Introduction

The horn fly, *Haematobia irritans* (L.), is an economically important hematophagous parasite of cattle. Adult flies spend most of the time resting on the host and females leave the host for short intervals to oviposit in fresh dung pats (McLintock and Depner 1954). Consequently, horn flies have been controlled effectively by applying insecticides on the hair coat of the host. A number of methods have been developed and used to deposit insecticide residues on cattle. Insecticides have been applied as sprays, dips, pour-ons, in self-treating devices such as dust bags, backrubbers and oilers, and more recently, in sustained-release devices such as insecticidal ear tags. The trend in the development of these methods has been a reduction in the amount of insecticide applied and cattle handling (Drummond 1985).

Development of insecticidal ear tags revolutionized the control of horn flies. Producers were offered a simple, easy and reliable method that controlled susceptible horn flies for the whole season with a single application (Ahrens and Cocke 1979; Knapp and Herald 1981; Schimdt and Kunz 1980; Williams and Westby 1980). Pyrethroid ear tags are based on homogeneous monolithic systems in which the insecticide is dissolved in a rate controlling polymeric matrix (Miller et al. 1983a). Insecticidal ear tags release a continuous but slowly declining amount of insecticide

(Miller et al. 1983a, 1986). Residues deposited on various regions of cattle by ear tags vary in time and space due to differences in insecticide release rate, rate of spread and degradation on hair coat (Miller et al. 1983a, 1986; Taylor et al. 1987; Yeung et al. 1989). These rates are influenced by a number of factors such as rubbing against other cattle and inanimate objects, licking, hair loss, degradation and environmental factors (Miller and Berry 1970; Roberts and Chamberlain 1963). Taylor et al. (1984, 1985) found different concentrations of residues in flank hair following application of cypermethrin ear tags in the U.K. and attributed the differences to environmental factors. Miller et al. (1986) showed that the release rate of fenvalerate and permethrin was higher in hot and humid than in cool and drier regions in the U.S.. Yeung et al. (1989) determined residues on the hair of calves in Canada following application of one fenvalerate tag per calf in a winter feedlot and two fenvalerate tags per calf under summer, dryland range conditions. However, for calves kept under summer, dryland range conditions, residues were determined at 30 days after application of tags only. In this study, I report residue levels deposited by fenvalerate and permethrin ear tags on cows following application of two tags per cow and one tag for every other cow under summer, pasture conditions in Manitoba, Canada. The tag treatments were chosen not only to simulate tagging recommendations for

control of face flies and horn flies (Anonymous 1987), but also to simulate actual tagging rates used by producers in Manitoba. In a survey conducted among cattle producers in 1987, 59% were applying one tag per animal, 37% less than one tag per animal and 2% were applying 2 tags per animal (Chapter III, Section I). Most producers (>95%) in each tagging category achieved satisfactory horn fly control.

Experimental Methods

Chemicals

Analytical standards of fenvalerate, *cis*- and *trans*-permethrin (Ag. Canada, Canada Centre for Pesticide Analytical Standards, Ottawa, Canada) were used. The internal standard of 3(4-chlorophenoxy)benzyl *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate was synthesized at the Agriculture Canada Research Station, Lethbridge, AB (Yeung et al. 1988). Redistilled 2,2,4-trimethyl pentane (Caledon Laboratories Ltd., Georgetown, ON Canada) was used for extraction. Acetone (Fisher Scientific, Nepean, ON) was used to rinse hair clipper blades between clippings.

Cattle

A cow-calf beef herd of mixed breeds at the University of Manitoba Glenlea Research Station was divided into four groups of 30 to 34 cows and their calves. Cows assigned to

group 1 received two Bovaid® (8% w/w fenvalerate, Ciba Geigy (Canada)) tags, while cows in group 2 received one Bovaid tag for every other cow. Group 3 cows received two Atroban® (10% w/w permethrin, Coopers Agropharm, Inc. (Canada)) tags per animal; group 4 had one Atroban tag for every other cow. All groups were tagged on 1 June in 1987. Groups 1 and 3 were tagged on 30 May and groups 2 and 4 on 22 June in 1988. The groups were kept in separate pastures and were rotated among pastures, depending on forage and weather conditions.

Hair Sampling

Five animals in groups 1 and 3, and 10 animals from groups 2 and 4, five with tags and five without, were sampled. Hair samples were collected from the head and neck, body (ventral side) and rump (behind the pin bone) regions of the same animals throughout the study. Hair was clipped with an electric hair clipper (Stewart Animal Clipper, Model ASC-35C with size 40 blades, Sunbeam Appliance Co., Toronto, Canada) powered by a generator. The blades were rinsed with acetone between clippings. Hair samples were wrapped in aluminium foil and put in plastic bags. The samples were transported to the laboratory and stored at -32 °C. Samples were collected on day 14, 28, 56, and 84 after tag application from all groups in 1987, and on day 16, 28, 56, and 84 from groups 1 and 3, and on day 19, 33 and 61 from groups 2 and 4 following tag application in

1988.

Extractions

Two 0.1 gram sub-samples of hair were taken from each sample and weighed in culture tubes. The samples were extracted with 3 mL of 2,2,4-trimethyl pentane on a wrist action shaker (Model 75, Burrel Corporation Pittsburgh, PA., USA) for 20 min and centrifuged at 2000 rpm for another 20 min. A 0.5 mL aliquot was mixed with an equal volume of 1 μ g of internal standard. One 2 μ L aliquot was taken from each tube and analyzed by gas chromatography (GC).

Gas Chromatography

Gas chromatography was performed on a 30 m x 0.53 mm i.d. DB-5 mega bore capillary column (Chromatographic Specialties Ltd., Brockville, ON, Canada) installed in a Hewlett Packard GLC model 5890 equipped with a ^{63}Ni electron capture detector (ECD). Injections were made in the splitless mode with 24 s delay. An initial oven temperature of 200 °C was held for 2 min, then increased at 30 °C min⁻¹ to 280 °C and held for 8 min. The injector port and detector temperature were maintained at 210 °C and 350 °C, respectively. The carrier gas was helium flowing at 9 mL min⁻¹ and makeup gas for the ECD was 95% argon + 5% methane flowing at 45 mL min⁻¹. Head pressure was maintained at 69 kPa.

Detector signals were acquired by a computer with Notebook Software (Laboratory Technology Corp., Wilmington, DE., USA.). The programme was used to compute the retention time, area and height of each peak. The peaks were quantified by comparing to the internal standard peak.

Standard Curve

The standard curves for *cis*- and *trans*-permethrin and fenvalerate were prepared by serial dilutions of a stock solution of analytical standards. 0.5 mL of 1 μ g of internal standard was mixed with respective 0.5 mL of diluted standards to give 2.5, 2, 1.5, 1.0, 0.5, 0.1 and 0.05 μ g of standard. Each concentration was replicated three times. A 1 μ L aliquot was injected into the GC. The height of each peak in the standard was divided by the peak height of the internal standard. A regression line fitted to peak height ratio data was used to quantify residues on hair.

Recoveries

Recoveries were determined by spiking 0.1 g of untreated hair with varying μ L of either fenvalerate or permethrin standard to give three concentrations of 2, 1 and 0.1 μ g. Each concentration was replicated three times. The samples were air dried and left in a fume cupboard for 24 hr. Spiked hair samples were extracted in the same manner

as field samples.

Data analysis

Mean residue levels were compared using the Restricted Maximum Likelihood (REML) option in PROC VARCOMP (SAS Institute Inc. 1985). Residue levels on each sampling day were compared within a treatment and body region. Comparison between treatments and regions were made within each sampling day and region, and within treatment and sampling day, respectively.

Results

Recovery studies

Acceptable recoveries were obtained at all three spiking levels of fenvalerate and permethrin (Table 3.3.1). Fenvalerate recoveries ranged from 89 to 104% at the 0.1 to 2 μ g fortification levels. Recoveries for *cis*- and *trans*-permethrin from fortified hair samples ranged from 96 to 97% and 96 to 104%, respectively. Minimum detection levels were below 15 pg for fenvalerate and 10 pg for *cis*- and *trans*-permethrin.

Residue levels on hair

In 1987, cattle that received one permethrin tag (group 4) lost most of them (Chapter III, Section IV). Seventeen per cent of the tags were lost during the first 2 weeks and about 50% by day 28. Consequently, hair samples from this

group were excluded from the analysis. In 1988, only two tags were lost in group 3 (Chapter III, Section IV).

The ratios of the two diastereoisomers (%SR,RS:%SS,RR) of fenvalerate and %*cis*-%*trans*-permethrin were similar throughout the study (Table 3.3.2). The two peak heights of each pyrethroid were thus combined to give total concentrations (Tables 3.3.3 and 3.3.4).

Residues deposited by two fenvalerate tags on hair from the head and body regions during the first 28 days following application of tags were significantly higher ($P < 0.05$) than those found on days 56 and 84 in 1987 (Table 3.3.3).

Fenvalerate residues on hair from the rump of cattle with two fenvalerate were statistically similar ($P > 0.05$) on all sampling days in both 1987 and 1988. Hair from the head of cattle with two fenvalerate tags had higher residues on day 14 post tagging than on day 28, but residues on days 56 and 84 were not significantly different ($P > 0.05$) in 1988.

Residues on hair samples within each region of cattle that received one fenvalerate or permethrin tag and those without tags in the same group did not differ ($P > 0.05$) during the sampling period in 1987 and 1988. Cattle that received two permethrin tags had higher residues on the hair from the head on day 14 in 1987 ($P < 0.05$) than on other sampling days but residues on days 28 and 56 did not differ significantly ($P > 0.05$) (Table 3.3.3). Concentrations of permethrin on hair from the body of cattle with two tags collected on days

14, 28 and 56, were similar ($P>0.05$); residues on the rump were comparable throughout the sampling period in 1987 (Table 3.3.3). In 1988, cattle with two permethrin tags had lower residues on day 16 than on day 28. The reduction in residues may be attributable to 12 mm of rain that fell during the three days preceding the sampling day. Sampling was postponed from day 14 to day 16 because the pastures were waterlogged. However, rain did not reduce the residues on hair from the head of cattle that had two fenvalerate tags, although residues on the body and rump of these cattle were reduced (Table 3.3.3). The concentration of pyrethroids found on hair of cattle on days 14 and 84 following tag application declined by 80 to 86% on the head, 73 to 78% on the body and 36 to 84% on the rump in 1987. A similar pattern was not observed in 1988 because of initial low residues on most regions. Concentration profiles of pyrethroids on hair from various regions of the treatments varied in 1987 and 1988, except for the residue profile on hair from the head of cattle with two fenvalerate tags which was similar in both years.

Generally, residues on the hair from the head of cattle with two tags were higher than residues on either body or rump during the first 4 weeks in both 1987 and 1988 ($P<0.05$) but residue levels were similar on all regions thereafter (Table 3.3.4). The concentration of fenvalerate and permethrin did not differ significantly ($P>0.05$) among

regions within each sampling date for cattle with one tag and those without a tag (Table 3.3.4). Concentrations of pyrethroids on hair from the head were higher on cattle with two tags than on cattle with one tag or without a tag in the first 4 weeks but not afterwards (Table 3.3.3). Residues on the body of cattle with two tags per animal were higher than residues on cattle with one tag or without a tag during the first 14 days in 1987 but not in 1988 (Table 3.3.4).

Discussion

Residues found on cattle hair following application of insecticide impregnated ear tags declined and varied over time. Although the pattern of decline was not consistent among the regions sampled, certain generalizations can be made. Residues on the head (near the site of attachment) of cattle with two tags declined rapidly and at a linear rate while residues on the rump declined at a slower rate. Residues on the head of cattle with two tags were significantly higher than residues on the body and rump during the first month but not thereafter. Cattle with or without a tag in the same group, on the other hand, had similar residues on all the regions on each sampling day ($P > 0.05$). There may be a concentration effect whereby regions with higher concentrations lose the insecticide at a faster rate to areas with low concentrations. The rate decreased as the residues declined or as the rate of residue

release from the tag decreased. After residues on the head of cattle with two tags (day 56) dropped to levels found on day 14 on cattle with one tag, no differences were observed within the regions.

Rate of residue dissipation following tag application can not be easily determined because residues are being deposited continuously, at a decreasing rate over time (Miller *et al.* 1983a). Decline of residues is therefore, not only a function of the initial residues and decay rates but also of the amounts that are continuously being deposited. Rate of insecticide release and levels of residue on hair are affected by many factors such as formulation and environmental factors with unpredictable effects. Yeung *et al.* (1989) suggested that ear tags either released more insecticide in the winter than in summer or the residues were being degraded faster in summer. In contrast, higher residues were deposited on the flank hair of cattle in summer than in winter (Taylor *et al.* 1984, 1985). Miller *et al.* (1986) reported that ear tags released more insecticide under hot and humid conditions than under cooler and drier conditions. In other studies, ear tags released about 19% of the insecticide over 140 days (Ahrens and Cocke 1979), and 30% of the insecticide over 115 days (Haufe 1982). At Glenlea, residues on hair were generally higher in 1987 than in 1988. Mean daily temperatures during the study were similar in both years (18.7 °C in 1987 versus

21.2 °C in 1988) but the mean daily precipitation differed (2.4 mm in 1987 versus 1.4 mm in 1988). However, total precipitation may not be as important overall as when rain fell relative to the sampling day. In 1988, residues on cattle with two permethrin tags were reduced on hair taken on day 16, following three days of rain. By day 28, there were no significant differences in residues observed in 1987 and 1988. Similar effects by rain on residues were reported by Holbrook (1986); Hopkins *et al.* (1985) and Young *et al.* (1985). Pyrethroids are highly lipophilic with low solubility in water (Elliot *et al.* 1978); the effect of rain on residues on hair is therefore most likely through physical abrasion. Holbrook (1986) reported significantly reduced residue levels on the upper body of cattle compared to lower portions following rainfall.

The mode of spread of insecticide on cattle hair following tag application is not fully understood. Similar levels of residues were found on various regions of tagged and untagged cattle in groups 2 and 4. Pyrethroids have high boiling points and low vapour pressure, and are not systemic (Elliott *et al.* 1978; Ruigt 1985); they are not likely to spread through the vapour phase to untreated animals or through the blood stream to other regions. The most likely mode of spread to untagged cattle is through direct contact with tagged cattle. Once on the animal, the insecticide, being lipophilic, spreads to other regions by

diffusion via the oily sebaceous secretions on the skin (Taylor et al. 1985). Other activities such as tail flicking and head movements can distribute residues to other parts of the body (Beadles et al. 1977; Stendel 1986; Taylor et al. 1985). The highest residues were detected on the head and body on the first sampling day, 14 days following tag application and declined thereafter. This is in agreement with other studies (Taylor et al. 1987; Yeung et al. 1989). During the first 2 weeks, the release of insecticide from the tag and deposition on hair were probably greater than the dissipation of residues on hair. Residues on the rump fluctuated with no consistent pattern. In most treatments, residues were higher on the rump than on the body but this was not statistically significant (Table 3.3.4). This may be due to more residues being deposited on the rump through tail flicking in addition to diffusion through sebaceous secretions, or it may be due to an artifact of sampling. Cattle were squeezed through a chute; some cattle may have transferred residues from the head region onto the rump of other animals. Alternatively, residues could have been transferred to the rump through cows mounting each other as the study was conducted during the breeding season.

Insecticide residues on cattle can be lost through the actions of rainfall, solar radiation, rubbing and licking, tail flicking, hair shedding, contact with the ground and

degradation by microorganisms (Miller and Berry 1970; Roberts and Chamberlain 1963). These factors affect residues on different regions of the body. Roberts and Chamberlain (1963) found that the tail removed insecticides from the rump and hind legs; licking removed residues behind the front legs while contact with the ground reduced insecticides on the belly and legs. Rainfall removes insecticide on areas incident to rain drops (Holbrook 1986). Solar radiation and microorganisms are expected to have similar effects on residues on most regions. Although the residues declined during the study, the ratio of isomers of fenvalerate and permethrin were fairly consistent during the study. Pyrethroid isomers degrade at preferential rates, with the *trans*-isomer being degraded faster (Gaughan and Casida 1978; Holmstead et al. 1978a, b; Lee et al. 1987; Ruzo 1982). The consistent isomeric composition observed in this study could be attributed to either similar rates of degradation of both isomers or to preferential degradation of one isomer followed by isomerization of the other to the equilibrium. The latter alternative seems likely, because *trans*-isomers of pyrethroids degrade faster on plants than the *cis*-isomers (Furuzawa et al. 1986; Gaughan and Casida 1978; Miyamoto and Mikani 1983), and the *cis*-isomer photochemically isomerizes to the *trans*-isomer more readily than vice versa (Furuzawa et al. 1986). Thus, the *trans*-isomer of permethrin and the RS,SR-fenvalerate may be

degraded faster on cattle hair, and the *cis*-permethrin and RR,SS-fenvalerate may then be photochemically isomerized to *trans*-permethrin and RS,SR-fenvalerate, respectively, to maintain the equilibrium.

The declining but persistent residues on cattle following application of tags have been identified as a major contributing factor to the pyrethroid resistance problem in horn flies (Sheppard et al. 1989; Sparks et al. 1985). Enough residues were transferred to untagged cattle to control susceptible horn flies within 14 days and were maintained for 84 days. This confirms findings in other studies where complete horn fly control was achieved on untagged cattle in a treated herd (Harvey and Brethour 1981, 1983; Knapp and Herald 1984; Sheppard 1980; Williams and Westby 1982) and by Holbrook (1986) who reported no differences in mortality of *Culicoides variipennis* (Coquillett) after exposure to hair from cattle with or without a tag in the same pen and hair from either side of cattle with one tag. In this study, no significant differences in fly numbers were observed among the treatments (Chapter III, Section IV). Control of horn flies in the groups that had two tags and one tag for every other animal was similar. Partial herd treatment does not provide refugia for susceptible horn flies and can not delay the development of resistance (Georghiou and Taylor 1977b). Whereas partial herd treatment can lead to preferential

survival of resistant flies (McDonald et al. 1987a; Roush et al. 1986) and behavioural resistance (Byford et al. 1987b), application of two pyrethroid tags per animal for horn fly control may not be economical and beneficial because residues on cattle with two tags were only superior during the first 28 days. Consequently, tagging rate may have little effect on rate of development of pyrethroid resistance in horn flies which is mainly due to an incompletely recessive gene (Byford et al. 1985; McDonald and Schmidt 1987; Roush et al. 1986). Increasing the tagging rate to four tags per animal does not kill all heterozygous and resistant horn flies (McDonald et al. 1987a). Furthermore, increasing tagging rate in the absence of effective immigration of susceptible flies into the population may lead to rapid fixation of the resistance genes in the population (Tabashnik and Croft 1982; Taylor and Georgiou 1979). Thus, the major factor responsible for rapid development of resistance in horn flies is the persistent but declining residues deposited by tags which eliminate susceptible flies in a treated herd. A tag for every other animal released enough residues to control susceptible flies. Low tagging rates may delay the development of resistance by allowing more heterozygous flies to survive.

Table 3.3.1. Recoveries of fenvalerate and permethrin from fortified hair of cattle.

Chemical	Fortification level ($\mu\text{g}/0.1\text{g}$ hair)	% Mean Recovery $\pm$ SD ^a
Fenvalerate	0.1	89.3 $\pm$ 3.5
	1.0	104.1 $\pm$ 4.0
	2.0	98.3 $\pm$ 1.1
<i>cis</i> -Permethrin	0.1	97.5 $\pm$ 9.2
	1.0	95.7 $\pm$ 8.6
	2.0	97.0 $\pm$ 5.4
<i>trans</i> -Permethrin	0.1	98.6 $\pm$ 7.0
	1.0	96.4 $\pm$ 6.0
	2.0	103.8 $\pm$ 9.7

^aMean of three replicates.

Table 3.3.2. Isomeric composition of fenvalerate (%SR,RS:%SS,RR) and permethrin (%trans:%cis) residues on hair of cattle following application of impregnated ear tags.

Group	Tags/animal	1 ^{1,2}	2	3	4
1987					
1	Two fenvalerate	58.0:42.0	58.8:41.2	57.2:42.8	58.0:42.0
2	One fenvalerate	58.0:42.0	57.2:42.8	60.3:39.7	60.5:39.5
2	No tag	58.3:41.7	59.0:41.0	60.0:40.0	56.5:43.5
3	Two permethrin	63.3:36.7	62.3:37.7	62.5:37.5	63.6:36.4
1988					
1	Two fenvalerate	58.8:41.2	59.4:40.6	59.2:40.8	59.6:40.4
2	One fenvalerate	54.0:46.0	57.8:42.2	50.9:49.1	
2	No tag	61.2:38.8	55.2:44.8	56.6:43.4	
3	Two permethrin	61.0:39.0	62.1:37.9	62.3:37.7	63.3:36.7
4	One permethrin	61.8:38.2	64.5:35.5	58.2:41.8	
4	No tag	61.5:38.5	67.0:33.0	57.0:43.0	

¹Sampling period 1, 2, 3, and 4 were 14, 28, 56 and 84 days following tagging in 1987, respectively. All groups tagged on 1 June 1987.

²Sampling period 1, 2, 3 and 4 were 16, 28, 56 and 84 days following tagging for group 1 and 3, and 19, 33 and 61 days following tagging for group 2 and 4, respectively. Groups 1 and 2 were tagged on 30 May 1988; groups 2 and 3 were tagged on 22 June 1988.

Table 3.3.3. Pyrethroid residues ($\mu\text{g g}^{-1}$) on hair from various body regions of cattle following tag application at Glenlea Research Station, Manitoba in 1987 and 1988. Sampling days are compared within each region and year.

Group	Tags/animal	Head				Body				Rump			
		1 ^{1,2}	2	3	4	1	2	3	4	1	2	3	4
1987													
1	Two fenvalerate	14.93ay ³	12.34ax	4.69by	2.69bx	7.06ax	5.32abx	1.80bx	1.71bx	4.65axy	4.22ax	6.36ax	2.97ax
2	One fenvalerate	4.75az	2.36ay	2.09ay	0.59ax	1.82ay	1.04ax	0.63ax	0.45ax	4.82axy	1.96ax	1.13ay	0.76ax
2	No tag	1.55az	0.90ay	0.78ay	0.30ax	1.51ay	0.53ax	0.53ax	0.33ax	1.10ay	0.78ax	0.74ay	0.57ax
3	Two permethrin	21.31ax	12.16bx	10.82bx	3.50cx	7.27ax	3.24ab	3.77abx	1.92bx	6.30ax	3.97ax	6.16ax	1.62ax
1988													
1	Two fenvalerate	13.82ax	8.34bx	3.22cy	1.39cx	1.67ax	3.29axy	3.00ay	0.76ax	1.62ax	3.54axy	2.76ay	1.06ax
2	One fenvalerate	2.48ay	1.10ay	0.66ay	- ⁴	0.65ax	0.63ay	0.49ay	-	0.80ax	0.92ay	0.38ay	-
2	No tag	1.72ay	0.76ay	0.31ay	-	0.65ax	0.89ay	0.53ay	-	0.87ax	0.87ay	0.80ay	-
3	Two permethrin	4.56by	10.75ax	7.44abx	4.05bx	1.78bx	5.27abx	6.98ax	1.76bx	1.31bx	4.35bx	10.96ax	1.75ax
4	One permethrin	4.69ay	1.03by	1.88aby	-	1.28ax	0.75ay	0.83ay	-	0.85ax	0.91ay	0.47ay	-
4	No tag	1.80ay	1.26ay	0.72ay	-	1.17ax	0.65ay	0.56ay	-	1.37ax	1.32axy	1.35ay	-

¹Sampling period 1, 2, 3, and 4 were 14, 28, 56 and 84 days following tagging in 1987, respectively. All groups were tagged on 1 June 1987.

²Sampling period 1, 2, 3 and 4 were 16, 28, 56 and 84 days following tagging for group 1 and 3, and 19, 33 and 61 days following tagging for group 2 and 4, respectively. Groups 1 and 3 were tagged on 30 May 1988; groups 2 and 4 tagged on 22 June 1988.

³Means followed by same letter within a row and region (abc) and within a column and year (xyz) are not significantly different ($P > 0.05$).

⁴No hair samples collected.

Table 3.3.4. Pyrethroid residues ($\mu\text{g/g}^{-1}$) on hair from various body regions of cattle following application of insecticidal ear tags at Glenlea Research Station, Manitoba in 1987 and 1988. Body regions are compared within each sampling day and year.

Group	Tags/animal	1 ^{1,2}			2			3			4		
		Head	Body	Rump	Head	Body	Rump	Head	Body	Rump	Head	Body	Rump
1987													
1	Two fenvalerate	14.93a ³	7.06b	4.65b	12.34a	5.32b	4.22b	4.69ab	1.80b	6.36a	2.69a	1.71a	2.97a
2	One fenvalerate	4.75a	1.82a	4.82a	2.36a	1.04a	1.96a	2.09a	0.63a	1.13a	0.59a	0.45a	0.76a
2	No tag	1.55a	1.51a	1.10a	0.90a	0.53a	0.78a	0.78a	0.53a	0.74a	0.30a	0.33a	0.57a
3	Two permethrin	21.31a	7.27b	6.30b	12.16a	3.24b	3.97b	10.82a	3.77b	6.16b	3.50a	1.92a	1.62a
1988													
1	Two fenvalerate	13.82a	1.67b	1.62b	8.34a	3.29b	3.54b	3.22a	3.00a	2.76a	1.39a	0.76a	1.06a
2	One fenvalerate	2.48a	0.65a	0.80a	1.10a	0.63a	0.92a	0.66a	0.49a	0.38a	- ⁴	-	-
2	No tag	1.72a	0.65a	0.87a	0.76a	0.89a	0.87a	0.31a	0.53a	0.80a	-	-	-
3	Two permethrin	4.56a	1.78ab	1.31b	10.75a	5.27b	4.35b	7.44b	6.98b	10.96a	4.05a	1.76a	1.75a
4	One permethrin	4.69a	1.28b	0.85b	1.03a	0.75a	0.91a	1.88a	0.83a	0.47a	-	-	-
4	No tag	1.80a	1.17a	1.37a	1.26a	0.65a	1.32a	0.72a	0.56a	1.35a	-	-	-

¹Sampling period 1, 2, 3, and 4 were 14, 28, 56 and 84 days following tagging in 1987, respectively. All groups were treated on 1 June 1987.

²Sampling period 1, 2, 3 and 4 were 16, 28, 56 and 84 days following tagging for group 1 and 3, and 19, 33 and 61 days following tagging for group 2 and 4, respectively in 1988. Groups 1 and 3 were treated on 30 May; Groups 2 and 4 on 22 June.

³Means followed by same letter within a row and period are not significantly different ($P > 0.05$).

⁴No hair samples taken.

CHAPTER III

SECTION IV

DYNAMICS OF PYRETHROID RESISTANCE IN A HORN FLY, *HAEMATOBIA*
IRRITANS (L.) (DIPTERA: MUSCIDAE), POPULATION ON TAGGED
CATTLE AT GLENLEA RESEARCH STATION, MANITOBA.

Abstract

Fenvalerate and permethrin tags at two tags per animal and one tag for every other animal provided nearly 100% control of horn flies (*Haematobia irritans* (L.)) for 8 weeks in 1987 but not in 1988 at Glenlea Research Station, Manitoba. Fly numbers on cattle increased after week 8 post-treatment in 1987 and week 6 in 1988. At the end of the season, mean fly numbers on treatment groups ranged from 3.5 to 29 flies per animal side in 1987 and 67 to 77 flies per animal side in 1988. LC_{50} 's of the flies to fenvalerate and permethrin increased towards the end of the season but the slope of log concentration-mortality regression lines decreased. At the beginning of the 1988 season, LC_{50} 's to both compounds were lower compared to those observed at the end of 1987 season. When tags were applied in 1988, susceptible flies were eliminated from the population and the LC_{50} 's increased from 0.42 to 3.14 $\mu\text{g}/\text{cm}^2$ for fenvalerate and 2.06 to 10.76 $\mu\text{g}/\text{cm}^2$ for permethrin. Mortality observed at discrimination concentrations of fenvalerate (0.625 $\mu\text{g}/\text{cm}^2$) and permethrin (5 $\mu\text{g}/\text{cm}^2$) during the season decreased from 67.9 to 2.8% and 66.8 to 5.7%, respectively.

Introduction

The horn fly, *Haematobia irritans* (L.) is an important pest of pastured cattle (Drummond *et al.* 1981 ; Kunz *et al.* 1984). Control of this pest has been achieved by applying insecticides to the hair coat of cattle. Development of ear tags as an insecticide delivery system made control of horn flies more efficient and economical (Ahrens and Cocke 1979; Knapp and Herald 1981; Schmidt and Kunz 1980; Williams and Westby 1980). However, use of insecticidal ear tags has led to rapid and widespread development of resistance which resulted in reduced efficacy. Horn fly resistance to insecticides incorporated in ear tags appeared in the U.S. following 2-4 years of tag usage (Kunz and Schmidt 1985a; Quisenberry *et al.* 1984; Schmidt *et al.* 1985; Sheppard 1984). Before ear tags were introduced for horn fly control, there were few documented cases of insecticide resistance (Burns and Wilson 1963; Harris *et al.* 1966; McDuffie 1960). The continuous but declining release rate of insecticides by the ear tags combined with some biological and ecological attributes of horn flies, have been implicated as major factors that have contributed to rapid development of resistance in treated populations (Sheppard *et al.* 1989; Sparks *et al.* 1985).

Herein, I report changes in the susceptibility of a horn fly population at the University of Manitoba's Glenlea Research Station (97° 08'W; 49° 38'N) to fenvalerate and

permethrin, and associated loss of efficacy. Pyrethroid-impregnated ear tags were first used in 1978 in experimental trials. Tags were applied in four seasons between 1978 and 1986 (Table 3.4.1).

Materials and Methods

Animals and Treatments

A cow-calf beef herd of mixed breeds was divided into four herds of 30 to 34 cows and their calves. Treatments were assigned to the groups as follows: Group 1 received two Bovaid® (8% w/w fenvalerate, Ciba Geigy (Canada) Ltd.) tags per adult animal; Group 2 received one Bovaid tag for every other adult animal; Group 3 was tagged with two Atroban® (10% w/w permethrin, Coopers Agropharm, Inc. (Canada)) per animal; and Group 4 had one Atroban tag for every other animal. The Groups were kept in separate pastures and rotated among the pastures in accordance with forage availability.

Animals were tagged as they passed through the chute and separated into their respective treatment groups. For animals allocated to Groups 2 and 4, the first half to pass through the chute were tagged. Tags were applied to all Groups on 1 June, 1987. In 1988, Groups 1 and 3 were tagged on 30 May. The breeding programme necessitated keeping Groups 2 and 4 together; as a result they were tagged and separated on 22 June.

Horn fly Counts

Horn fly counts were taken on one side of the same five randomly selected cows for Groups 1 and 3 and 10 cows (five with and five without tags) for Groups 2 and 4 with the aid of binoculars. Pre-treatment fly counts were not taken in 1987. In 1988, pre-treatment fly counts were taken on 10 randomly selected cows. Fly counts were taken on 10 randomly selected animals in Groups 2 and 4, two weeks after Groups 1 and 3 were tagged. Tag retention among the Groups was also monitored biweekly on all tagged animals. Fly count data were transformed by $\sqrt{(X + 0.5)}$ before analysis using nested General Linear Model option in SAS (SAS Institute, Inc. 1985).

Bioassays

When there were sufficient numbers of flies on cattle, a composite sample of horn flies was collected from all Groups and evaluated for their susceptibility to fenvalerate and permethrin using the impregnated filter paper petri-dish method as described by Sheppard and Hinkle (1987). Each concentration was replicated twice and controls were run concurrently. Mortality was determined immediately after the flies were released into petri-dishes and two hours later. Flies that could not walk or fly were considered dead. Data were subjected to probit analysis using POLO-PC (Le Ora Software, Inc. 1987) to determine the LC_{50} 's and 95%

confidence limits (CL), and slopes of log concentration-mortality regression lines. Non-overlap of 95% CL was taken as the criterion of significant differences between LC_{50} 's. Likelihood ratio tests were used to test the hypothesis of parallelism and equality of regression lines ($\alpha=0.05$). A susceptible strain of horn flies was obtained from USDA, Kerrville, Texas as pupae and maintained in the laboratory (Chapter III, Section II) as a standard. Resistance factors (RF's) were calculated by dividing the LC_{50} of the field flies by the LC_{50} of the susceptible strain. Mortality was also noted at discrimination concentrations of $0.625 \mu\text{g}/\text{cm}^2$ for fenvalerate and $5 \mu\text{g}/\text{cm}^2$ for permethrin. These concentrations were immediately above concentrations in the bioassay range that killed 100% of susceptible flies.

Results

Tag retention

In 1987, no ear tags were lost except Group 4 (Table 3.4.2). Group 4 lost 17% of the tags during the first 2 weeks, and 70% were lost by week 10, representing a tag ratio of 1 tag per 3.4 adult animals. This tag loss cannot be explained as all animals were tagged in the same manner using the same applicators. In 1988, tag loss was minimal. Only Group 3 lost two tags during the first 2 weeks and there was no further tag loss (Table 3.4.2).

Horn fly counts

All treatments provided 100% control of horn flies for at least 8 weeks in 1987, except for Group 4 (Table 3.4.3 and Fig. 3.4.1). A few horn flies (less than one horn fly per animal) were observed after two weeks on animals without tags in Group 4 and four weeks on tagged animals in the same Group. Horn flies were observed on other Groups in week 10 post-treatment. Horn fly numbers continued to increase such that at the end of the experiment (week 13 post-treatment), there were more than 10 flies per side for Groups 1, 2 and 4, but less than four flies per side of each animal in Group 3. There were no differences ($P>0.05$) in the number of horn flies on cattle with two fenvalerate tags per animal and one fenvalerate tag per two animals (Table 3.4.3 and Fig. 3.4.1).

In 1988, there were approximately 30 flies per side of each animal before treatment. Two weeks post-treatment, fly numbers were reduced but not eliminated on Groups 1 and 3 that received 2 tags per animal. Fly numbers were similar to pre-treatment levels on untagged Groups 2 and 4. Four weeks post-treatment for Groups 1 and 3 and less than a week post-treatment for Groups 2 and 4, fly numbers per animal side were lower though not significantly so ($P>0.05$) on Groups that received fenvalerate tags compared to Groups that received permethrin tags (Table 3.4.3 and Fig. 3.4.2). Numbers of horn flies in all treatments continued to

increase to over 67 flies per animal side after 12 weeks post-tagging, when the experiment was terminated. There were no differences ($P>0.05$) in the numbers of horn flies among the treatment Groups 6 weeks post-treatment.

Bioassays

When flies were tested near the end of 1987 season (24 August and 3 September), there was a slight increase in the resistance factors to both fenvalerate and permethrin (Table 3.4.4). The log concentration-mortality regression lines of flies collected during the two sampling dates towards the end of the season were parallel for fenvalerate and permethrin and the slopes declined (Table 3.4.4).

Resistance factors observed in spring of 1988 were lower than those observed at the end of 1987 season for both compounds (Table 3.4.4). There was an increase in resistance factors following tag application. The LC_{50} 's of pre-treatment and first post-treatment flies collected in the eighth week in 1988 were significantly different based on non-overlap of 95% confidence limits. Other 95% confidence limits for adjacent sampling dates overlapped. Resistance factors to fenvalerate increased from 3 to 22-fold, while resistance factors to permethrin increased from 1.6 to 8.2-fold. A general increase in the slopes of the regression lines was observed in 1988 (Table 3.4.4).

Mortality at both discrimination concentrations of

fenvalerate and permethrin decreased at the end of 1987 season (Table 3.4.5). High mortality in response to both compounds was observed in the pre-treatment sample collected in 1988 compared to that collected at the end of 1987 season. However, there was a substantial decrease in mortality to both compounds after tags were applied. Mortality at discrimination concentrations remained low for the rest of the season.

Discussion

Previous insecticidal ear tag treatments at Glenlea Research Station afforded nearly 100% control of the horn fly population (Galloway *et al.* 1983, 1986). This level of control was not achieved in 1987 and 1988, and therefore resistance was suspected.

The rate of resistance development depends mainly on the original resistance gene frequency, its dominance and selection pressure (proportion killed) (Comins 1977). Dominance of resistant genes depends on the dose of insecticide applied and the resistance of heterozygous individuals relative to homozygous resistant and susceptible individuals (Curtis *et al.* 1978; Georghiou and Taylor 1977a; Tabashnik and Croft 1982; Wood and Mani 1981). A higher dose kills all heterozygous and some resistant homozygotes, making a resistance gene functionally recessive. With immigration of susceptible individuals into the treated

population, the few resistant homozygotes that survive the treatment will mate with susceptible immigrants producing heterozygotes that can be killed by the next treatment, thereby delaying the development of resistance (Georghiou and Taylor 1977a, b; Tabashnik and Croft 1982; Wood and Mani 1981). A lower dose on the other hand, kills only the homozygous susceptible individuals. More individuals carrying the resistance gene are left in the population to contribute to the next generation and resistance develops rapidly (Georghiou and Taylor 1977a; Tabashnik and Croft 1982; Wood and Mani 1981). However, without immigration of susceptible individuals into the treated population, dose levels have little influence on the development of resistance (Comins 1977; Georghiou and Taylor 1977a, b; Tabashnik and Croft 1982). Rate of development of resistance will be determined by the rate at which susceptible individuals are removed from the population (Tabashnik and Croft 1982). In the case of insecticidal ear tags, resistance genes are rendered both functionally recessive and functionally dominant because the amount of insecticide deposited on the hair of cattle from the ear tags declines during the season (Chapter III, Section III; Miller et al. 1983a, 1986; Taylor et al. 1985, 1987; Yeung et al. 1989). At the beginning of the season when ear tags are releasing higher doses, resistance is functionally recessive but later in the season, resistance becomes

functionally dominant as the residues fall below a threshold that is lethal to most heterozygotes. This is substantiated by the appearance of horn flies on all Groups in week 10 post-treatment in 1987 and increases in fly numbers after week 6 post-treatment in 1988. Perhaps levels of residues non-lethal to heterozygotes were attained after week 8 in 1987 and week 6 in 1988. However, this is unlikely because residues on hair of cattle in 1987 and 1988 were similar (Chapter III, Section III). Alternatively, the population was probably becoming more resistant; heterozygotes tolerated higher residues as the resistance gene was being integrated into the genetic background of the population. McDonald and Schmidt (1990) reported that higher shifts in dose-mortality regression lines were observed when the genetic background of the heterozygous horn flies was from more resistant parents than when it was from the susceptible ones. Survival of heterozygous individuals on tagged cattle seems to be critical and probably the major factor in the rapid development of resistance to insecticides in ear tags. Similar observations were made by McDonald *et al.* (1987a). Survival rates of heterozygous horn flies on tagged cattle were higher than those of homozygous susceptible flies even after 1 week post-treatment. Survival rates of heterozygous flies at Glenlea should be lower than those reported by McDonald *et al.* (1987a) because their heterozygous flies came from a cross with highly resistant flies that were 100-

fold resistant at LC_{50} level. In the field, early in the development of resistance, survival of heterozygous flies would be expected to occur towards the end of the season as residues on the hair coat decline.

There is very little effective immigration of susceptible flies into tagged herds (Sparks *et al.* 1985) because ear tags give 100% control of susceptible flies throughout the season (Ahrens and Cocke 1979; Galloway *et al.* 1983, 1986; Schmidt and Kunz 1980; Williams and Westby 1980). Residues on cattle hair at the end of the season are still sufficient to kill susceptible immigrants, hence the few flies observed on tagged cattle in this study were probably resistant immigrants or residents. This is supported by mortality observed at discrimination concentrations. The proportion of resistant individuals in the first sample collected in week 12 post-tagging (24 August 1987) was slightly lower than those in the second sample collected in week 13 (3 September 1987). In spring of 1988, the frequency of individuals resistant to fenvalerate decreased from about 82% to 32% and 45% to 33% for individuals resistant to permethrin. A similar decline in resistance during winter has been reported by Sheppard *et al.* (1989) and Weinzierl *et al.* (1990). The decline in resistance observed between fall and spring is likely not due to immigration of susceptible individuals into the population because residues on cattle hair coat are still

sufficient to kill susceptible immigrants, but due to gene segregation and recombination in the population. The spring population is composed of the progeny of resistant and/or heterozygote individuals that survived lower residues deposited by tags at the end of the season. The spring population was not exposed to residues on cattle hair at the end of the season before diapause. The spring population, therefore, included some susceptible individuals which are offspring of heterozygous individuals. More evidence of resistance gene segregation and recombination could have been obtained if fly samples were collected from dung pats using emergence traps. Fly samples used in the bioassays were biased towards resistant individuals that survived residues on hair because flies were collected from tagged cattle after susceptible flies had been eliminated from the population.

During the early stage of resistance development, some of the progeny of flies observed on cattle are still somewhat susceptible, and these would be killed by residues on cattle as evidenced by the slow increase in numbers of flies on cows following tagging in 1987. During this period, the genetic background would be progressively reconstructed towards greater fitness and survival on tagged cattle. The gene segregation and reconstruction is further supported by decline in slopes of regression lines towards the end of 1987, and general increase in slopes of

regression lines in 1988. In 1987 and previous years, resistance genes might have been segregating in the population with few resistant individuals in the population. The fly population would be reduced to a sufficiently low level that the population loses its capacity to sustain positive growth. The capacity is further reduced by higher residues deposited by ear tags early in the season but would improve as residues decline at the end of the season. In 1988, the resistance gene was becoming fixed in the population (the population was becoming more homogeneously resistant), and hence the observed loss in efficacy and increase in slopes of regression lines.

Horn fly resistance to pyrethroids is mainly due to a single major incompletely recessive gene similar to knock down resistance (*kdr*) (Byford *et al.* 1985; McDonald and Schmidt 1987; Roush *et al.* 1986). The *kdr* gene is intrinsically, incompletely recessive. Only homozygous individuals are fully resistant and heterozygotes are not fully susceptible but close to homozygous susceptible individuals. The flies observed on cattle at the beginning of the season after tagging, when residues are high, are expected to be homozygous for resistance. These individuals may be at a disadvantage at first and their contribution to the next generation will be small resulting in slow increase in population size. But as the residues decline, less resistant heterozygous flies begin to survive. This

increases the proportion of individuals carrying the resistance gene in the population, which contribute to overall population growth. This stage might have been reached after week 8 post-treatment in 1987 and week 6 post-treatment in 1988 when an increase in fly numbers was observed on cattle.

At discrimination concentrations in 1987, 65% and 15% of the flies collected in week 12 post-treatment were resistant to fenvalerate and permethrin, respectively. A week later, over 80% were resistant to fenvalerate and 45% were resistant to permethrin. In 1988, after tags were applied, the proportion of individuals in the population resistant to fenvalerate increased to more than 90% in week 8 post-treatment from a pre-treatment frequency of 32%, and remained high for the rest of the season. The frequency of flies resistant to permethrin increased from 33% before treatment to 66% in week 8, and 95% in weeks 10 and 12 post-treatment. Prior to week 8 in 1988, fly numbers were low probably because of the interaction between gene segregation and concentration of residues on hair. Between weeks 1 and 8, there might have been few individuals carrying the resistance gene in the population because residue levels might have been above a threshold that is lethal to most heterozygotes. But by week 8, the residues on most Groups had declined by over 48% (Chapter III, Section III), and most heterozygotes were probably able to survive and

reproduce. Resistant individuals eventually became numerous in the population. A parallelism test was accepted for adjacent sampling dates, an indication that log concentration-mortality regression lines were steadily shifting towards higher concentrations during the selection process. It appears that each season tags are applied, there is a progressive increase in the frequency of resistant individuals and ability to tolerate higher residues on hair.

Insecticidal ear tags selected for resistance rapidly in this study. At first the selection process was slow and nearly 100% reduction in flies was achieved. During this phase of resistance development, the frequency of resistant individuals in the population was too low and the fly population was too small to sustain significant population growth with the exclusion of susceptible immigrant flies. This is consistent with the findings by Georghiou and Taylor (1977a) who reported that population size increased slowly when the resistance gene was recessive. Once a few flies were observed on tagged cattle, the frequency of resistant individuals was high enough to sustain positive growth and resistance developed rapidly in the absence of effective immigration of susceptible flies into the population, resulting in loss of control. This might explain why pyrethroid impregnated ear tags failed after 2 to 4 yr of use (Quisenberry *et al.* 1984; Schmidt *et al.* 1985; Sheppard

1984). Technically, resistance in horn flies at Glenlea Research Station appeared after 5 yr of intermittent tag use. Resistance was expected to evolve slowly in Manitoba where the fly season is about four months compared to the southern U.S. where the fly season is over seven months (more generations per season) (Georghiou and Taylor 1977a, b; Tabashnik and Croft 1982). It appears all that is required is a few flies carrying the resistance genes for effective selection for resistance to insecticides in ear tags to occur, and for the frequency of the genes in the population to increase. Development of resistance may not be delayed by delaying tagging or removing tags before the onset of diapause because the susceptible flies that have been preserved are killed by ear tags leaving resistant flies to interbreed. Development of resistance may be delayed by switching to other products with different modes of action or control strategies before the frequency in resistance genes is high in the population, that is, when a few flies are observed on tagged cattle. Resistance management strategies will succeed if selection pressure is reduced or withdrawn before resistant individuals proliferate to the point where control failure is noticeable.

Table 3.4.1. History of pyrethroid ear tag use on cattle at Glenlea Research Station, Manitoba.

Year	Treatment
1978	Fenvalerate tags
1979	Fenvalerate tags
1980	Not treated
1981	Not treated
1982	Not treated
1983	Flucythrinate tags
1984	Not treated
1985	Not treated
1986	Cypermethrin tags

Sources: Galloway 1979; Galloway and Adams 1978; Galloway and Cantor 1981; Galloway and Paget 1980; Galloway et al. 1982, 1983, 1986, pers. comm.. Cattle received one tag per animal.

Table 3.4.2. Percentage of tag loss in beef herds at Glenlea Research Station, Manitoba.

Group	Tags/animal	Week ¹						
		2	4	6	8	10	12	13
1987 ²								
1	Two fenvalerate	0	0	0	0	0	0	0
2	½ fenvalerate	0	0	0	0	0	0	0
3	Two permethrin	0	0	0	0	0	0	0
4	½ permethrin	17.6	47.0	53.0	53.0	70.6	70.6	70.6
1988 ³								
1	Two fenvalerate	0	0	0	0	0	0	
2	½ fenvalerate	NT ⁴	0	0	0	0	0	
3	Two permethrin	3.6	3.6	3.6	3.6	3.6	3.6	
4	½ permethrin	NT	0	0	0	0	0	

¹Week 1 is week including 1 June; Week 0 represents pretreatment fly counts.

²All groups tagged on 1 June 1987.

³Groups 1 and 3 tagged on 30 May; Groups 2 and 4 tagged on 22 June.

⁴Not treated

Table 3.4.3 Mean horn fly counts per side of tagged beef herds at Glenlea Research Station, Manitoba.

Group	Tags/animal	Week ¹							
		0	2	4	6	8	10	12	13
1987 ²									
1	Two fenvalerate	NC ³	0	0	0	0	1.2	6.8	11.5
2	One fenvalerate	NC	0	0	0	0	3.2	6.2	11.5
2	No tag	NC	0	0	0	0	1.6	4.4	12.5
3	Two permethrin	NC	0	0	0	0	2.0	3.4	3.5
4	One permethrin	NC	0	0.4	0.2	0.2	14.2	3.4	16.8
4	No tag	NC	0.4	0.2	0.2	0	20.8	10.0	28.8
1988 ⁴									
1	Two fenvalerate	28.3	0.8	1.0	10.4	39.2	69.2	72.5	
2	One fenvalerate	28.2	28.8	0.2	3.0	21.0	52.2	67.5	
2	No tag	29.8	26.6	0.6	3.0	30.6	77.5	72.5	
3	Two permethrin	29.8	6.4	18.6	7.0	38.0	72.5	72.6	
4	One permethrin	28.2	28.8	18.4	9.4	25.8	66.6	74.9	
4	No tag	29.8	26.6	16.4	9.2	39.2	80.0	77.5	

¹Week 1 is week including 1 June; Week 0 represents pretreatment fly counts.

²All groups tagged on 1 June 1987.

³No counts taken.

⁴Groups 1 and 3 tagged on 30 May; Groups 2 and 4 tagged on 22 June.

Table 3.4.4. Responses of horn flies at Glenlea Research Station to fenvalerate and permethrin, 1987 and 1988.

Compound	Date	n	LC ₅₀ (95% CL) ¹	Slope (SE) ²	RF ³
Fenvalerate	24/08/87	76	0.77 (0.59-1.76)	3.77 (1.24)	4.2
	03/09/87	102	0.92 ⁴	1.64 (0.61)	5.3
	13/06/88	138	0.42 (0.22-0.61)	1.95 (0.40)	2.3
	25/07/88	87	1.40 (0.93-1.95)	2.05 (0.51)	7.7
	08/08/88	343	2.07 (1.69-2.56)	1.71 (0.17)	11.5
	22/08/88	278	3.14 (2.38-4.23)	2.89 (0.29)	17.4
Permethrin	24/08/87	176	2.16 (1.83-2.60)	3.04 (0.41)	1.7
	03/09/87	120	3.25 (2.43-4.87)	2.01 (0.51)	2.5
	13/06/88	192	2.06 (1.28-2.95)	1.49 (0.28)	1.6
	25/07/88	118	6.85 ⁴	1.47 (0.45)	5.2
	08/08/88	245	15.58 (11.79-28.4)	2.74 (0.57)	11.9
	22/08/88	158	10.76 ⁴	4.10 (0.79)	8.2

¹µg/cm².

²Slope (standard error) of log concentration mortality line.

³Resistance Factor = LC₅₀ of field-collected flies divided by LC₅₀ of susceptible laboratory strain.

⁴Data too heterogeneous to calculate 95% CL; g > 0.4 (Le Ora Software Inc. 1987).

Table 3.4.5. Mortality observed at discrimination doses of fenvalerate and permethrin in a horn fly population from Glenlea Research Station, Manitoba.

Date	Week ¹ post- treatment	% Mortality at 0.625 µg/cm ² of fenvalerate	RF ²	% Mortality at 5 µg/cm ² of permethrin	RF
24/08/87	12	35.4	2.8	85.2	1.2
03/09/87	13	18.1	5.5	55.1	1.8
13/06/88	0	67.9	1.5	66.8	1.5
25/07/88	8	8.6	11.6	33.6	3.0
08/08/88	10	11.8	8.5	4.8	20.8
22/08/88	12	2.8	36.0	5.7	17.7

¹All groups tagged on 1 June 1987. Groups 1 and 3 tagged on 30 May; Groups 2 and 4 tagged on 22 June in 1988.

²Resistance Factor = Mortality of susceptible flies (100%) divided by mortality of field-collected flies.

Figure 3.4.1. Mean number of horn flies per side of tagged cattle at Glenlea Research Station, Manitoba, 1987. All groups tagged on 1 June 1987.

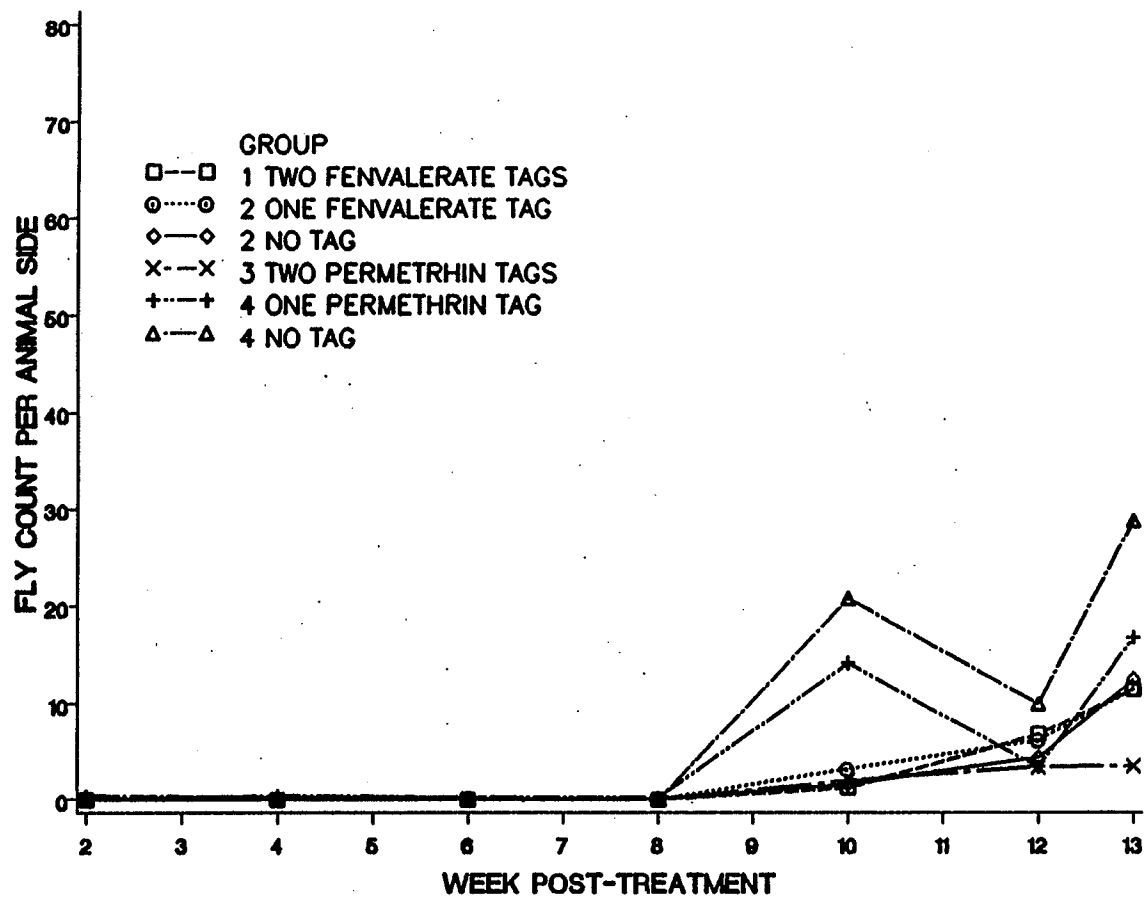
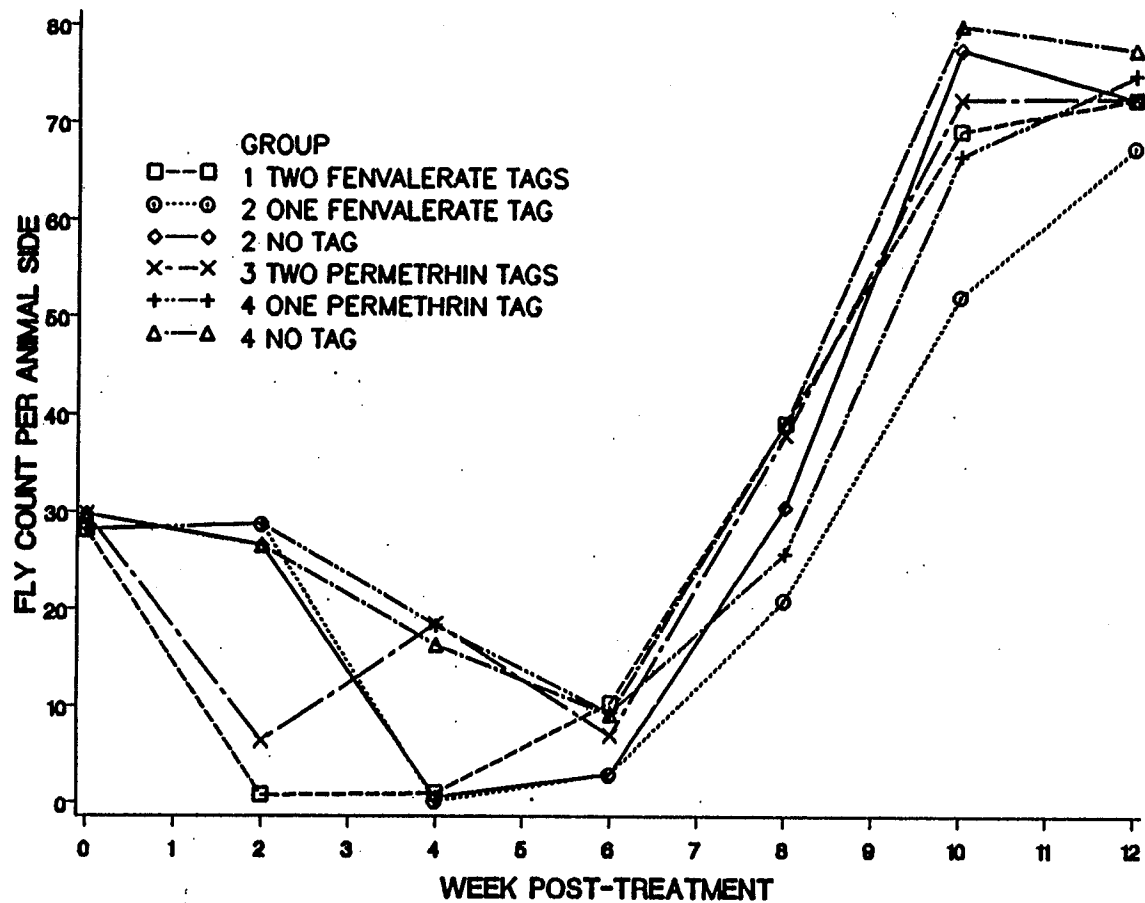


Figure 3.4.2. Mean number of horn flies per side of tagged cattle at Glenlea Research Station, Manitoba, 1988. Groups 1 and 3 tagged on May 30; Groups 2 and 4 tagged on 22 June.



CHAPTER III**SECTION V**

**STABILITY OF PYRETHROID RESISTANCE IN TWO HORN FLY,
HAEMATOBIA IRRITANS (L.), POPULATIONS IN MANITOBA IN THE
ABSENCE OF SELECTION PRESSURE.**

Abstract

Reversion of resistance was monitored in two pyrethroid resistant horn fly populations in the absence of selection pressure in Manitoba, Canada. Although there was a decrease in LC_{50} 's observed in fall 1988 and spring 1989, resistance to fenvalerate and permethrin was stable during the season in both populations. Slopes of the log concentration-mortality regression lines were less than 2 on most occasions, an indication that the populations were heterogeneous, consisting of both susceptible and resistant flies. Abstaining from pyrethroid-impregnated ear tag use for one season after they fail is not long enough for substantial reduction in resistance levels to occur. Based on these results, alternation of insecticides within a season appears to be a more effective strategy to manage pyrethroid resistance in horn flies than alternating insecticides among years or using mixtures of insecticides.

Introduction

Development of insecticide resistance by insects is an evolutionary phenomenon brought about by differential survival and reproduction of susceptible and resistant individuals following application of insecticides (Metcalf 1983; Roush and McKenzie 1987). Because insect populations are not resistant to insecticides before selection, resistance alleles are assumed to be rare and associated with deleterious effects in untreated populations (Crow 1957). In the absence of insecticide pressure, the deleterious effects associated with resistance alleles are expected to reduce the frequency of resistant alleles in a population (Abedi and Brown 1960). Although there are some exceptions (Argentine *et al.* 1989; Mwangala 1982; Roush and Plapp 1982), reversion of resistance in the absence of selection pressure is the basis for some of the resistance management tactics (Georghiou 1983; Leeper *et al.* 1986; Sawicki and Denholm 1987). Comins (1984) suggested that it is possible, in principle, for insecticides to have cycles of useful life if populations revert to susceptibility when the insecticides are withdrawn.

Because resistance factors in resistant horn fly populations usually decrease between fall and spring (Chapter III, Section IV; Sheppard 1987), I determined whether the decline in resistance observed between fall and spring continues during the season in the absence of

selection pressure in two resistant horn fly populations at the University of Manitoba Glenlea Research Station and Libau Community Pasture.

Materials and Methods

Horn flies were collected from cattle at the Glenlea Research Station biweekly and occasionally at a herd at the Libau Community Pasture in June to September 1989. Flies were exposed to a range of concentrations of technical fenvalerate (Ciba Geigy, Canada) and permethrin (Coopers Agropharm, Inc. Canada) on filter papers in petri-dishes (Sheppard and Hinkle 1987). Mortality was assessed immediately after the flies were introduced into the petri-dishes and 2 hours later. Flies that could not fly or walk were considered dead.

Data were analyzed using a probit analysis programme (POLO-PC; Le Ora Software, Inc. 1987). Concentrations that killed 50% of the flies (LC_{50} 's) were considered significantly different if their 95% confidential limits (CL) did not overlap. Non-overlap of the slopes $\pm$ S.E. was the criterion used to determine significant changes in slopes.

Results

The LC_{50} 's of flies from Glenlea to fenvalerate were similar (overlap of 95% CL) throughout the season (Table 3.5.1). Responses of the flies from Glenlea to permethrin at LC_{50} 's were similar except for flies collected on 5 September that were more tolerant than flies collected on 1 and 15 August.

Slopes for the log concentration-mortality regression lines varied among sampling dates. Flies collected on 1 August had the highest slopes for both fenvalerate and permethrin. The parallelism hypothesis was rejected for dose-mortality regression lines of flies from Glenlea for fenvalerate. However, the hypothesis was accepted when the flies collected on 1 August were removed from the analysis. Regression lines for flies from Glenlea exposed to permethrin were parallel.

The LC_{50} 's and the slopes of the regression lines for flies from Libau Community Pasture did not differ significantly for either fenvalerate or permethrin (Table 3.5.1).

Discussion

Resistance management tactics are aimed at reducing the frequency of resistant individuals in a population either by killing individuals carrying resistance genes or conserving susceptible individuals in a population (Leeper et al. 1986;

Georghiou 1983; Roush 1989; Sawicki and Denholm 1987).

Resistance management strategies have been classified into prophylactic and remedial categories (Brown 1981; Sawicki and Denholm 1987). Strategies under the former category are applied before the frequency of resistant individuals is high enough to impair control and aim to keep the frequency of resistant individuals below economic levels (Brown 1981; Sawicki and Denholm 1987). These include rotating insecticides with different modes of action across space (mosaics) or over time (alternation), using mixtures of insecticides that do not confer resistance to each other insecticide, and minimizing selection pressure through timed application of non-persistent insecticides, avoidance of slow-release formulations and preservation of refugia (Georghiou 1983; Roush 1989). Remedial strategies are applied once the frequency of resistant individuals in a population reaches economical frequencies and resistance is expressed through impaired control (Brown 1981; Sawicki and Denholm 1987). These strategies are used to restore the efficacy of the insecticide by reducing the frequency of resistant individuals to the original frequency. They include sequential use of insecticides which lack cross-resistance after one insecticide fails, and use of mixtures which consist of the failing insecticide and additives that enhance its efficacy, for example, synergists or other insecticides (Brown 1981; Georghiou 1980, 1983). The

rationale behind most of these tactics is that the frequency of resistant individuals will decline when the selecting insecticide is withdrawn (Georghiou 1980, 1983; Roush 1989). However, for this to occur, individuals carrying resistance genes should be at a reproductive disadvantage and less fit (Georghiou 1983).

In the two horn fly populations evaluated in this study, resistance to fenvalerate and permethrin appeared to be stable. The LC_{50} for flies from Glenlea for fenvalerate at the beginning of the season in June, 1989 was lower than that observed in fall, 1988 (Chapter III, Section IV). Subsequent LC_{50} 's in 1989 were comparable to those observed in 1988 (Chapter III, Section IV), so resistance did not decline when tags were withdrawn. The response of flies from Glenlea to permethrin in 1989 was similar to 1988 when tags were applied (Chapter III, Section IV). There were no significant changes in the responses of flies at Libau to fenvalerate and permethrin in 1989 compared to 1988 (Chapter III, Section II). This is consistent with the findings by Weinzierl *et al.* (1990), who reported that two years abstinence from using pyrethroid-impregnated ear tags was insufficient to allow an adequate decline in resistance levels.

Stability of pyrethroid resistance in these horn fly populations could be due to either absence of susceptible flies immigrating into the populations to dilute the

resistance genes or to no differences in the fitness between resistant and susceptible flies. The first is unlikely because the herds were untreated therefore, there were no barriers to effective immigration of susceptible flies. The second could result through coadaptation, a process in which resistance genes are incorporated into the genetic background (Roush and McKenzie 1987). Abedi and Brown (1960) reported that resistance to DDT in *Aedes aegypti* (L.) declined in the absence of DDT, but after three cycles of selection and relaxation, resistance to DDT stabilized. Similar observations were made by McEnroe and Naegele (1968) in *Tetranychus urticae* (Koch). Selection for resistance was negatively correlated with fitness until the resistance genes were integrated into the genetic background. It is possible that the two horn fly populations examined in this study had already passed beyond a point where resistance genes are disadvantageous.

The rate at which resistances decline depends on the intrinsic fitness ratio of various genotypes which is a measure of the strength of regression to susceptibility in the absence of the selecting insecticide (May and Dobson 1986). Although the various fitness components such as fecundity, developmental rates, viability and mating competitiveness among the various genotypes were not measured in this study, it appears that resistant horn flies suffered no disadvantages. The slopes of the regression

lines were low (less than 2 on most occasions), an indication that the population was probably heterogeneous including both susceptible and resistant individuals. Pyrethroid resistance in horn flies is mainly through an insensitive target site (*kdr*) (Byford *et al.* 1985; McDonald and Schmidt 1987; Roush *et al.* 1986) which also confers resistance to DDT (Farnham 1977; Miller *et al.* 1983b). DDT resistance in Danish house fly populations persisted for more than 20 years (Keiding 1977). If this is the case for horn flies, then a year of pyrethroid abstinence as in this study or two years (Weinzierl *et al.* 1990) is not long enough for any substantial decline in resistance levels to occur.

When there are no disadvantages associated with the resistance gene, rotating insecticides across time (alternation) is more likely to forestall or reduce resistance levels than mixtures or rotating insecticides in space (mosaics) (Curtis 1987; Roush 1989). Byford *et al.* (1987c) favoured mosaics for suppressing resistant horn flies. They argued that mosaics can function as either a mixture or an alternation of insecticides if exchange between sectors occurs within a generation or between generations, respectively. However, for mosaics to function as a mixture or an alternation, the insects must be highly mobile and the size of the sectors must be related to the biology of the insect e.g. distance and direction of

dispersal. Mosaics will mainly function as unplanned alternations because most of the horn flies that disperse are newly emerged ones (Chamberlain 1982; Guillot et al. 1988; Tugwell et al. 1966) and their dispersal is indiscernible (Kunz et al. 1983; Skoda et al. 1987). Mosaics will, therefore, exacerbate the resistance problem in horn flies as some generations may be selected with the same insecticide continuously. In this case, the use of mosaics is tantamount to sequential rotation of insecticides and is inadvisable because it may lead to rapid fixation of resistance genes in a population. This has been stressed as the major cause of resistance to pyrethroid-impregnated ear tags in horn flies (Sheppard et al. 1989; Sparks et al. 1985).

Resistance in horn flies can be suppressed by using planned alternations of insecticides with different modes of action. This has been done successfully against pyrethroid resistant *Heliothis armigera* (H.) in Australia and Zimbabwe (Sawicki 1989; Sawicki and Denholm 1987) and against *H. virescens* (F.) in the U.S. (Riley 1989). Pyrethroid applications are restricted to defined periods in a season and the selection pressure is restricted to one or two generations in a year. In Australia, the frequency of pyrethroid resistant larvae has been maintained at less than 10% at the beginning of each season (Forrester and Cahill 1987). Pyrethroid resistance in horn flies can also be

managed by alternating pyrethroids with organophosphorus insecticides, carbamates (Byford *et al.* 1985, 1988) or avermectin (Galloway *et al.* 1990). Pyrethroids can still be included in the alternations as sprays or in dust bags and backrubbers even where pyrethroid-impregnated ear tags have failed. Horn fly populations that were uncontrollable with pyrethroid-impregnated ear tags have been controlled with pyrethroid applied as sprays (Sheppard 1987) or in dust bags or backrubbers (Harvey and Brethour 1986). Use of these insecticides should be restricted to certain generations per season. This will entail withdrawal of slow release devices which apply selection pressure over many generations in favour of methods that apply insecticides intermittently such as sprays. Dust bags and backrubbers can release insecticide intermittently if they are charged with small quantities of insecticide that will last for a short period. They can also be charged with different insecticides in a season in an alternation. Resistant individuals not killed by one insecticide will most likely be killed by the next insecticide. Consequently, at the beginning of each season, there will be few individuals in the population carrying resistance genes to the first insecticide. The success of this strategy does not depend on reversion of resistance in the absence of the first insecticide but on the proportion of individuals resistant to the first insecticide that are killed by the second

insecticide. If the two insecticides are equally effective in controlling both the susceptible horn flies and horn flies resistant to the other insecticide, then it is possible to forestall resistance and avoid selecting doubly resistant individuals.

This strategy will definitely require compliance and co-operation from cattle producers and insecticide manufacturers, and may be difficult to implement in countries that have free market economies. Producers should accept some inconvenience or time consuming methods at the expense of lengthening the useful life of the few insecticides available on the market while insecticide manufacturers should be content with reduced returns on their investment in the short run.

Table 3.5.1. Responses of resistant field populations of horn flies to fenvalerate and permethrin in the absence of selection pressure in Manitoba, 1989.

Location	Date	Fenvalerate				Permethrin			
		n ¹	LC ₅₀ ²	95% CL	Slope ± S.E. ³	n	LC ₅₀	95% CL	Slope ± S.E.
Glenlea	16/6/89	78	0.74 ⁴	-	1.44±0.89	69	9.95 ⁴	-	1.83±0.99
	04/7/89	200	2.31	1.37-3.27	2.24±0.37	172	6.90	4.96-10.07	1.55±0.26
	18/7/89	185	2.31	1.65-3.23	1.41±0.23	172	8.42	5.92-13.83	1.39±0.24
	01/8/89	151	1.59	1.25-1.97	2.84±0.39	152	5.43	4.21-7.08	2.21±0.32
	15/8/89	278	1.38	0.68-2.21	1.59±0.21	298	6.15	4.73-8.30	1.67±0.19
	05/9/89	286	2.92	1.37-5.76	1.15±0.22	324	14.58	9.75-28.37	1.37±0.26
Libau	15/6/89	83	3.04	1.99-13.48	2.32±0.43	-	-	-	-
	03/7/89	296	2.66	0.67-15.69	1.78±0.56	320	14.86	10.98-23.19	1.21±0.82
	24/7/89	205	2.87	2.27-3.68	1.93±0.52	250	10.78	7.13-19.02	1.27±0.78

¹Number of flies treated (total of two replicates)

²µg/cm²

³Slope and Standard error of log concentration mortality lines.

⁴Data too heterogeneous to calculate 95% CL; g > 0.4 (Le Ora Software Inc. 1987).

CHAPTER III**SECTION VI**

**EFFICACY OF VARIOUS TAG FORMULATIONS CONTAINING DIAZINON AND
 λ -CYHALOTHRIN AGAINST PYRETHROID-RESISTANT HORN FLY,
HAEMATOBIA IRRITANS (L.), POPULATIONS IN MANITOBA, CANADA.**

Abstract

Tag formulations containing 20% diazinon, 4% cypermethrin + 18% diazinon, 10% λ -cyhalothrin, and 10% λ -cyhalothrin + 13% piperonyl butoxide were evaluated for their efficacy against pyrethroid-resistant horn flies, *Haematobia irritans* (L.), in Manitoba, Canada. All tags were effective; there were fewer than five horn flies per animal side on most occasions. One 4% cypermethrin + 18% diazinon tag per animal was as effective as two tags per animal for the duration of the experiment (105 days). There was no difference in the control afforded by combination tags containing either 4% cypermethrin + 18% diazinon or 10% λ -cyhalothrin + 13% piperonyl butoxide and single insecticide tags containing either 20% diazinon or 10% λ -cyhalothrin. Tags containing λ -cyhalothrin were generally more effective than diazinon tags. The pre-treatment LC_{50} 's of bioassayed fly populations were 0.4 to 3.4 $\mu\text{g}/\text{cm}^2$ for fenvalerate and 1.1 to 17.0 $\mu\text{g}/\text{cm}^2$ for permethrin.

Introduction

Pyrethroid-impregnated ear tags have become the main method for horn fly control in North America since their introduction in the late 1970's and early 1980's. The rapid acceptance of ear tags by producers has been attributed to ear tags' cost effectiveness and ease of application (Ahrens and Cocke 1979; Kunz and Schmidt 1985a). However, accompanying widespread acceptance of ear tags has been the development of pyrethroid resistance in horn fly populations (Kunz and Schmidt 1985a, Quisenberry *et al.* 1984; Schmidt *et al.* 1985; Sheppard 1984; Sparks *et al.* 1985). Pyrethroid resistance in horn flies was first reported in 1982 (Kunz and Schmidt 1985a) and has since been reported in most of U.S. (Kunz and Schmidt 1985a; Marchiondo 1987).

When resistance to an insecticide develops in a population, there are few options available to producers. Producers can either increase the dosage of insecticide applied or switch to another insecticide (Georghiou 1980). In the case of horn fly control with pyrethroid ear tags, increasing tagging rates may not succeed in controlling resistant horn flies (Chapter III, Section IV; McDonald *et al.* 1987a). The most effective option for producers is to switch to other insecticides. The success of this option depends on the availability of alternative insecticides. Given the limited number of insecticides available commercially for horn fly control in Canada, it is important

to evaluate more insecticides to increase producers' choices.

The purpose of this study was to evaluate the effectiveness of various ear tag formulations in controlling suspected or known pyrethroid-resistant horn fly populations on pastured cattle in Manitoba, Canada.

Materials and Methods

A total of 14 herds and four tag formulations were used in the study. Location of herds, tag formulations tested, tagging rates and dates are shown in Table 3.6.1. Organophosphorus (OP) tags and combination tags (OP + pyrethroid) were supplied by Ciba Geigy (Canada), and λ -cyhalothrin tags were supplied by Coopers Agropharm, Inc. (Canada). The herds were selected on the basis of 1988 resistance tests and producers' complaints concerning horn fly control problems with pyrethroid ear tags. All herds, with the exception of the control herd (Herd D) at Libau Community Pasture, were cow-calf herds. Herd D was composed of dry cows. At Lundar, tags were applied after fenvalerate and permethrin tags had failed to reduce horn flies on herds J and K, respectively. Only cows were tagged.

Horn fly counts were made on one side of 10 randomly selected cows each week, on most occasions. Flies from Libau Community Pasture, Narcisse Community Pasture, and Lundar were evaluated for resistance to fenvalerate and

permethrin using the impregnated filter paper method (Sheppard and Hinkle 1987) prior to tag application. The LC_{50} 's for the horn fly populations were determined with the probit option of POLO-PC (Le Ora Software, Inc. 1987).

Results

All tag formulations tested were highly effective in providing protection from horn flies throughout the season (Table 3.6.2). Combination tags (4% cypermethrin + 18% diazinon, and 10% λ -cyhalothrin + 13% piperonyl butoxide) were as effective as single insecticide tags for the duration of the trial. Tags containing λ -cyhalothrin were more effective than the diazinon tags, on average.

Mean fly counts were less than five flies per animal side on most occasions for all treatments (Table 3.6.2). Fly counts on herds C and J were greater than 40 flies per animal side on one occasion but no flies were observed in the following week. There is no explanation for these observations.

Only flies from Libau Community Pasture, Narcisse Community Pasture and the two herds near Lundar were tested for susceptibility to fenvalerate and permethrin before tags were applied. The LC_{50} 's ($\mu\text{g}/\text{cm}^2$) of the flies for fenvalerate and permethrin were 3.4 and 17.0 for flies from Libau Community Pasture (herd D), 0.4 and 1.1 for flies from Narcisse Community Pasture (herd M), 2.8 and 16.1 for flies

from Lundar (herd J), and 2.0 and 16 for flies from Lundar (herd K), respectively.

Discussion

All tag formulations containing diazinon and λ -cyhalothrin tested in this study provided season-long control of pyrethroid-resistant horn flies. Similar results have been reported in other studies (Byford *et al.* 1987d; 1988; Cocke *et al.* 1990b; Sheppard and Marchiondo 1987). Tags containing 4% cypermethrin + 18% diazinon at both one tag per animal and 2 tags per animal, and 20% diazinon controlled pyrethroid-resistant horn flies. Tags containing either fenvalerate or permethrin had failed to control flies at herds J and K, respectively. Efficacy of the combination tag (4% cypermethrin + 18% diazinon) can be attributed to diazinon in the tag because horn flies resistant to fenvalerate and permethrin are cross-resistant to cypermethrin but not to organophosphorus insecticides (Bull *et al.* 1988; Byford *et al.* 1985; Byford and Sparks 1987; Sparks and Byford 1988). The efficacy of diazinon tags against pyrethroid-resistant horn flies observed in this study suggests that these populations are not cross-resistant to organophosphorus insecticides. Lack of cross-resistance between pyrethroid and organophosphorus insecticides can be exploited in the control of pyrethroid-resistant horn flies. Although there appears to be no

cross-resistance between pyrethroids and organophosphorus insecticides in horn flies, not all organophosphorus insecticides that are active in laboratory bioassays can be used successfully in ear tags for the control of pyrethroid-resistant horn flies probably because of incompatibility with the tag matrix (Byford and Sparks 1987; Byford et al. 1988). Among the organophosphorus insecticides evaluated in ear tags, diazinon and pirimiphos-methyl were suitable for use against pyrethroid-resistant horn flies (Byford and Sparks 1987; Cocke et al. 1990b; Sparks and Byford 1988), in addition to tetrachlorvinphos which is already registered in Canada. Dioxathion, stirofos and chlorpyrifos tags gave relatively poor control of pyrethroid-resistant horn flies (Byford and Sparks 1987; Byford et al. 1988). Thus, the choice of organophosphorus insecticides that can be used in ear tags for control of pyrethroid-resistant horn flies may be limited. Most of the organophosphorus insecticides can still be used against pyrethroid-resistant horn flies in dust bags and backrubbers (Harvey and Brethour 1986).

Tags containing λ -cyhalothrin alone or in combination with piperonyl butoxide were effective against pyrethroid-resistant horn flies. These results corroborate those found in other pyrethroid-resistant horn fly populations (Cocke et al. 1990b; Sparks and Byford 1988). Pyrethroid resistance in horn flies is largely due to an insensitive-target site (*kdr*) mechanism (Bull et al. 1988; Byford et al. 1985;

McDonald and Schmidt 1987; Roush et al. 1986). The *kdr* factor confers cross-resistance to many pyrethroids and DDT in many insect species (Miller et al. 1983b), including horn flies (Byford et al. 1985). This cross-resistance does not appear to extend to λ -cyhalothrin in horn fly populations evaluated in this study at concentrations released by the ear tags during the trial. Sparks and Byford (1988) observed no significant difference between the response of pyrethroid-resistant and susceptible horn fly strains to λ -cyhalothrin. Lower cross-resistance to λ -cyhalothrin has also been reported in tobacco budworm, *Heliothis virescens* (F.) (Campanhola and Plapp 1987; Leonard et al. 1988). However, one drawback concerning the use of λ -cyhalothrin tags is that their efficacy is accompanied by higher resistance levels to fenvalerate (Cocke et al. 1990b), an indication that λ -cyhalothrin selects for the same mechanism as fenvalerate. Levels of resistance observed in the populations evaluated in this study may not be high enough to overcome the higher activity of λ -cyhalothrin. Sparks et al. (1988) reported high levels of cross-resistance to λ -cyhalothrin in a strain of tobacco budworm that was highly resistant to cypermethrin. High levels of cross-resistance to cyhalothrin (composed of 4 stereoisomers) has also been demonstrated in pyrethroid-resistant tobacco budworm (Campanhola and Plapp 1989) and in the mosquito, *Culex quinquefasciatus* Say (Halliday and Georgiou 1985). Thus,

the efficacy of λ -cyhalothrin tags against pyrethroid-resistant horn flies may not be due to absence of cross-resistance but largely due to its greater biological activity (Ruigt 1985).

Use of insecticide mixtures has been suggested as one approach for managing resistance in pest populations (Georghiou 1980, 1983). The success of mixtures depends on three major assumptions: the mechanisms of resistance to components in a mixture should be different, the population under consideration should not be resistant to any of the components, and the components should have similar decay patterns (Georghiou 1983). Combination tags containing a pyrethroid and an organophosphorus insecticide meet the first criterion (Byford *et al.* 1985; Sheppard and Marchiondo 1987) but not the last two. Pyrethroid resistance in horn fly populations is already widespread (Chapter III, Section II; Kunz and Schmidt 1985a; Marchiondo 1987), and there are differences in the rates of release of insecticide from ear tags (Miller *et al.* 1983a, 1986) and in residues on different body regions (Chapter III, Section III; Taylor *et al.* 1987; Yeung *et al.* 1989). In addition, the efficacy and length of protection provided by pyrethroid ear tags against susceptible horn flies is generally superior to that obtained with organophosphorus tags (Ahrens and Cocke 1979; Sheppard 1983; Williams and Westby 1982).

Although tags containing a pyrethroid and an

organophosphorus insecticide were effective in controlling pyrethroid-resistant horn flies, caution should be exercised in their use. Tags containing pyrethroids increased pyrethroid resistance levels in horn fly populations (Cocke *et al.* 1990b). Use of combination tags containing a pyrethroid will only increase the pyrethroid resistance problem in horn flies and prevent any chances of resistance reversion. The efficacy of organophosphorus ear tags against pyrethroid-resistant horn flies may be short-lived. Horn flies have already demonstrated their propensity to develop resistance to organophosphorus-impregnated ear tags. Resistance to stirofos ear tags occurred after two seasons of use (Sheppard 1983). Therefore, as Sheppard *et al.* (1989) and Sparks *et al.* (1985) pointed out, the only solution to horn fly resistance problems lies in the reduction of selection pressure. This can be accomplished by using methods that can apply insecticide intermittently such as sprays, dust bags, oilers and backrubbers. Dust bags, oilers and backrubbers can either be charged with limited amounts of insecticides to last a few days or be removed from pastures to reduce the selection pressure. This will in turn allow effective immigration of susceptible flies into treated populations when the insecticides have run out or when the devices are removed from pastures. Furthermore, it is possible to alternate different insecticides within the season in these devices which is not

possible with current tag formulations.

Table 3.6.1. Location of herds and treatment schedule for various tag formulations in Manitoba, Canada - 1989.

Herd	Location	Tag formulation	Tags/ animal	Tagged week ending
A	Libau Community Pasture	4% cypermethrin + 18% diazinon	2	27/5/89
B	"	20% diazinon	2	"
C	"	4% cypermethrin + 18% diazinon	1	"
D	"	control	0	-
E	Libau	10% λ -cyhalothrin + 13% piperonyl butoxide	1	24/6/89
F	"	20% diazinon	2	10/6/89
G	"	20% diazinon	2	10/6/89
H	"	10% λ -cyhalothrin + 13% piperonyl butoxide	1	17/6/89
I	"	10% λ -cyhalothrin	1	10/6/89
J	Lundar	20% diazinon	2	24/6/89
K	"	10% λ -cyhalothrin + 13% piperonyl butoxide	1	24/6/89
L	Gunton	20% diazinon	2	27/5/89
M	Narcisse Community Pasture	20% diazinon	2	27/5/89
N	Narcisse	control	0	-

Table 3.6.2. Mean numbers of horn flies per animal side of tagged and untagged herds in Manitoba - 1989. See Table 1 for assigned treatments.

Week ending	Herd													
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
03/6/89	0.3	2.5	0.3	50.5	3.25	33.8	51.5	31.3	13.0	NC	NC	0	28.3	NC
10/6/89	0	1.0	2.3	70.0	6.9	1.0	2.0	45.3	1.3	NC	NC	2.3	4.5	61.3
17/6/89	0.8	0.8	1.8	131.3	3.0	0	0.8	0	0	NC	NC	6.0	1.3	41.5
24/6/89	0	0	9.8	552.5	2.3	7.6	NC	9.0	0	38.0	105.5	3.8	0.8	91.3
01/7/89	0.3	0.3	0	71.3	0	0.3	4.8	0.5	0	0	0	NC	0.3	70.8
08/7/89	0	0.3	0	63.0	0	0	4.0	4.0	0	0	0	1.5	0	110.5
15/7/89	0	0	1.5	128.8	0	0	2.8	0	0	0	0	0.3	1.5	238.8
22/7/89	0.3	0	0	99.3	0	0	2.0	0.5	0	0.3	0	0	0	340.0
29/7/89	0	0	8.8	90.3	0	0	0	1.8	0	0	0	0	0	177.5
05/8/89	0	0	44.0	NC	0	0	3.8	0	0	0	NC	0	0	NC
12/8/89	0	0	0	31.5	0	0	NC	0	0	0	0.4	0	0	NC
19/8/89	0	0	0	65.3	0	0	3.8	0	0	10.4	0.5	0	0	111.3
26/8/89	0	0.3	0	78.8	0	0	0	0	0	41.0	0	0	2.8	185.0
02/9/89	0.3	0	11.0	82.5	0	0.3	0.3	0	0	0	0	0	4.7	NC

NC - No fly counts taken.

CHAPTER IV

GENERAL DISCUSSION

This study was prompted by increasing reports of horn fly resistance to pyrethroids in the U.S.. In most cases, resistance was reported in 2 to 4 yr of tag use (Kunz and Schmidt 1985a; Quisenberry et al. 1984; Schmidt et al. 1985; Sheppard 1984). Ear tags were registered in Canada in 1981 but there were no confirmed reports of horn fly resistance to pyrethroids prior to this study. Thus, the first objective of this study was to determine the extent of insecticidal ear tag usage in Manitoba.

Two hundred cattle producers responded to a survey carried out in 1987 (Chapter III, Section I). This represented 40% of the total questionnaires that were distributed through Livestock Specialists. Seventy-two per cent of the respondents were beef producers while 22% were dairy producers and the remainder were mixed producers (both beef and dairy). Insecticidal ear tags were rapidly accepted by the majority of producers in Manitoba following their registration for fly control. The majority of producers (81%) who responded were using ear tags to control flies on their cattle. The remaining 19% producers were either using other control methods or not practising any control methods. Among tag users, 56% adopted ear tags within three years of their registration; another 44%

adopted tags after three years of registration. Beef and dairy producers equally adopted ear tags over other previously available methods for horn fly control.

Most of the producers who apply tags (59%) were following tagging recommendations for horn fly control (Anonymous 1987, 1989); another 37% applied less than one tag per animal. More than 95% of tag users were satisfied with the fly control tags were affording. Thus, despite using ear tags for a period (4 yr) similar to their counterparts in the U.S., most of Manitoba producers were still achieving satisfactory horn fly control with ear tags. This was expected from predictions of most computer simulation models on development of resistance (Comins 1977; Georghiou and Taylor 1977a, b; Wood and Mani 1981; Tabashnik and Croft 1982). Resistance develops rapidly in insect species with short generation time and many generations per year (Georghiou 1980, 1983; Georghiou and Taylor 1977a, b). In southern parts of the U.S., the generation time of horn flies is shorter and there are more generations per season than in Canada (Depner 1961; Hogsette and Ruff 1986; Kunz and Miller 1985a; Thomas and Kunz 1986). The next logical step was to undertake a field survey for resistance to verify the producers' reports.

Surveillance for resistance in pest populations is an important aspect of resistance management (Brown 1981). With an efficient bioassay method, it is possible to detect

changes in the susceptibility of pest populations and to determine whether such changes impair control in the field. Original susceptibility levels of pests to an insecticide can only be determined by either bioassaying pest populations before the insecticide is used in the field or using laboratory strains that have not been exposed to the insecticide. However, because of inbreeding in laboratory strains, concerns are usually raised about whether these strains still represent the wild susceptible ones after colonization in the laboratory as closed populations. The response to fenvalerate and permethrin of the susceptible laboratory strain from Kerrville, Texas, used in this study was similar to field flies collected from untreated herds. This strain can, therefore be used with confidence as a standard to document changes in susceptibility of horn fly populations in the field.

In agreement with producer responses, low levels of resistance and few resistant horn fly populations were encountered in 1987 (Chapter III, Section II). Most of the horn fly populations sampled in 1987 were not more than 3-fold resistant to fenvalerate and permethrin compared to the susceptible strain. Resistance levels below 3-fold did not appear to impair control as there were few flies on tagged cattle (Chapter III, Section IV). When resistance levels exceed 3-fold, resistance management programmes should be implemented to prevent the proliferation of resistance in

the population. As resistance levels approach 3-fold, it will not take long before treatment failure is noticed (Chapter III, Section IV). Although the sampling effort was not the same in each year, it can still be concluded that the number of resistant horn fly populations and intensity of the resistance increased during the study. Resistant populations were interspersed among susceptible ones throughout the province. Pyrethroid ear tags were still effective in controlling horn flies on some herds (Chapter III, Section II). Resistant populations were encountered in all the regions of Manitoba sampled. No samples were collected from the Northwest region, but there were unconfirmed reports of control problems with ear tags in 1990.

Resistant populations exhibited variable degrees of cross-resistance to the pyrethroids that were evaluated in this study. The populations were resistant to fenvalerate, permethrin and cypermethrin but not to λ -cyhalothrin (Chapter III, Section II, Section VI). Lack of cross-resistance to λ -cyhalothrin was probably due to the higher activity of the two isomers against insects (Ruigt 1985). Pyrethroid resistance in horn flies is largely due to the insensitive target site (*kdr*) mechanism (Bull et al. 1988; Byford et al. 1985; McDonald and Schmidt 1986, 1990; Roush et al. 1986) which confers cross-resistance to pyrethroids and DDT (Miller et al. 1983b). Tags containing λ -

cyhalothrin increased fenvalerate resistance levels in a resistant horn fly population (Cocke et al. 1990b), suggesting that λ -cyhalothrin selects for the same resistance mechanism. Thus, as horn flies become highly resistant to pyrethroids through continued use of pyrethroids, the *kdr* resistance mechanism is expected to extend to λ -cyhalothrin as demonstrated in a strain of tobacco budworm (Sparks et al. 1988).

This is the first intensive study in Canada to investigate pyrethroid resistance in horn fly populations. The distribution of resistant horn fly populations in North America extends to Manitoba and Saskatchewan. By the end of the 1989 horn fly season, there were no confirmed cases of resistance in other provinces of Canada. There has been no extensive work done on horn fly resistance in these provinces, including Saskatchewan, due to an acute shortage of veterinary entomologists addressing the problem. Surveillance for resistant horn fly populations in these provinces is based mainly on complaints from producers. Producer complaints can be an effective method of monitoring resistance when there is an effective extension network and when producers can positively identify insect pests. In the producer survey (Chapter III, Section I), most producers indicated that they were still achieving satisfactory horn fly control and follow up bioassays confirmed producer reports. In 1988, producer reports concerning horn fly

control failures were received and subsequent bioassays confirmed the existence of higher levels of resistance in the populations. In the absence of bioassay data, producer complaints can, therefore, be an alternative method of resistance surveillance where there is an effective extension service, although resistance will be detected only when the frequency of resistant flies is already high in the population.

Higher levels of resistance were observed in herds in the southern part of the Eastern region in 1989 (Chapter III, Section II). These observations, coupled with lack of evidence of horn fly resistance in most provinces of Canada and the pattern in which horn fly resistance spread in North America, led to the hypothesis that resistant horn flies might have been progressively dispersing north from the south. Horn flies were first thought to be restricted to certain herds as closed populations with little movement between herds (Chamberlain 1981, 1982; McLintock and Depner 1954). More evidence, however, indicate that horn flies can disperse over long distances (Beadles *et al.* 1975; Byford *et al.* 1987a; Kinzer and Reeves 1974). The speed with which horn flies infest cattle attests to the efficiency of their dispersal (Kunz *et al.* 1983; Skoda *et al.* 1987). When dispersing resistant horn flies land on treated herds, they interbreed with impunity because susceptible flies are continuously being eliminated by persistent residues on

tagged cattle (Chapter III, Sections III and IV; McDonald et al. 1987a; Roush et al. 1986). When the dispersing flies land on untreated herds, resistant alleles are swamped by the local susceptible horn flies, and the frequency of resistant flies in the population is kept at low levels. It is, therefore, possible to have pockets of highly resistant populations interspersed among untreated susceptible populations as in this study (Chapter III, Section II). The treated herds act as sinks for dispersing susceptible flies. As tags continue to be used, resistant horn flies are expected to spread to other provinces of Canada.

Studies conducted at Glenlea Research Station support results of most computer models on development of resistance. The dose of insecticide and immigration of susceptible individuals into treated populations have been implicated by most models as key determinants of resistance selection rates (Comins 1977; Georgiou and Taylor 1977a, b; Tabashnik and Croft 1982; Wood and Mani 1981). Another important factor is persistence of insecticide; persistent insecticides select for resistance rapidly because selection pressure continues even when the pest population declines below economic levels (Denholm et al. 1983; Taylor and Georgiou 1979). Pyrethroid-impregnated ear tags meet the requirements for rapid selection of resistance. In 1987, selection pressure was exerted on the population even when very few flies were observed on cattle for the first 8 weeks

following tag application (Chapter III, Section IV). The residues deposited by the tags on various regions of cattle declined slowly during the season (Chapter III, Section III). Based on previous studies at the station (Galloway et al. 1983, 1986), the residues found on cattle hair at the end of the season are still sufficient to kill susceptible flies. Ear tags, therefore selectively eliminate susceptible flies in the population, preventing any chances for dilution of resistance genes in the population by susceptible immigrants for the whole season. Furthermore, the declining but persistent residues, render the resistance genes both functionally recessive and dominant (Chapter III, Sections III and IV). Initially, the residues are high enough to kill most of the heterozygous resistant flies and some of the homozygous resistant flies. High residues observed shortly after tagging (Chapter III, Section III), render the resistance genes effectively recessive, resulting in a few highly resistant flies on cattle. As the residues decline, more heterozygous flies survive and resistance genes become functionally dominant. Heterozygous flies carry both the resistant and susceptible genes and are responsible for the decline in resistance factors observed between fall and spring (Chapter III, Section IV). When heterozygous individuals mate, some of their progeny will be susceptible according to the Hardy-Weinberg ratio. During the season, these susceptible flies are continuously being

killed by residues on cattle. At the end of the season, the susceptible flies overwinter without exposure to residues on cattle and emerge in spring. In the early stages of development of resistance, a large proportion of the overwintering generation will be susceptible. As tags are used continuously, the proportion of susceptible flies in the overwintering generation decreases as the resistance gene is being integrated into the background genome of the population. Delaying application of ear tags at the beginning of the season or removing tags towards the end of the season can be an effective method of delaying the development of resistance. Delaying tagging allows susceptible flies in the overwintering population to dilute resistance genes. Similarly, removing tags towards the end of the season increases the proportion of susceptible flies in the overwintering population. However, application of tags negates the benefits achieved by either delaying tagging or removing tags because ear tags eliminate susceptible flies leaving only resistant flies (Chapter III, Section IV). In 1988, mortality of the flies from Glenlea at discrimination concentrations decreased substantially following tag application and remained low for the rest of the season. Complete elimination of susceptible flies appears to play an important role in the development of resistance to pyrethroid-impregnated ear tags in horn fly populations.

Tagging rates (concentration of residues on hair) did not appear to influence the rate at which resistance developed. Residues on hair of cattle with two tags per animal were significantly higher ($P < 0.05$) than those on cattle with one tag for every other animal for the first month at most (Chapter III, Section III). Residues on both groups were not significantly different ($P > 0.05$) for the rest of the season (Chapter III, Section III). There were also no significant differences ($P > 0.05$) in the number of flies on cattle with two tags per animal and one tag for every other animal. Tagging rate can have an influence on the development rate of resistance when there is immigration of susceptible flies into treated populations (Comins 1977; Georgiou and Taylor 1977a, b; Tabashnik and Croft 1982). However, ear tags are still effective in controlling susceptible flies (including susceptible immigrants) for the whole season even at a tagging ratio of less than one tag per animal (Chapter III, Section IV; Harvey and Brethour 1981; Knapp and Herald 1984; Williams and Westby 1982). Thus reducing or increasing tagging rates may have little effect on the development of resistance in horn flies. A higher tagging rate may even select for resistance more rapidly without immigration because a higher proportion of susceptible and heterozygous flies will be eliminated from the population leaving only highly resistant flies to interbreed (Tabashnik and Croft 1982).

Pyrethroid resistance in two populations remained relatively stable in the absence of selection pressure (Chapter III, Section V). This has important implications for resistance management in horn flies. Most of the resistance management strategies that rely on resistance reversion in the absence of selection pressure cannot succeed in managing the pyrethroid resistance problem in horn flies. Among the resistance management strategies, alternation of insecticides with different modes of action within a season appears to be more expedient than mixtures or mosaics. Alternation of insecticides within a season will restrict selection pressure to a few generations within a season as opposed to alternation between years in which all generations in a season are subjected to one insecticide. If alternations within a season are well coordinated, then few generations will be selected with the same insecticide throughout the season. Because of the likelihood of long distance dispersal among horn fly populations, alternation of insecticides should cover a large area to avoid horn fly generations being selected with the same insecticide continuously. Alternation of insecticide within a season has been successful in managing pyrethroid resistance in cotton pests (Riley 1989; Sawicki 1989; Sawicki and Denholm 1987). The only important requirement in the alternation is that each insecticide in the alternation be equally effective against flies that are

susceptible and resistant to the other insecticide in the alternation.

The success of mixtures and mosaics depends on stringent requirements which cannot be met because of wide spread occurrence of pyrethroid resistance in horn fly populations and difficulty in predicting the extent and direction of dispersal, respectively. Furthermore, mixtures and mosaics can provide continuous selection pressure for the whole season, leading to rapid development of resistance, as has been the case with pyrethroid-impregnated ear tags. Tags containing diazinon or λ -cyhalothrin alone or as a mixture were effective in controlling pyrethroid-resistant horn flies (Chapter III, Section VI). The fact that these tags were effective is an indication that pyrethroid resistance levels observed in these populations do not confer cross-resistance to diazinon and λ -cyhalothrin. However, these tags are short term solutions to the pyrethroid resistance problem in horn flies because they have the same shortcomings as fenvalerate and permethrin tags. Resistance is likely to appear to diazinon and λ -cyhalothrin tags.

The best strategy in delaying or managing resistance in horn flies depends on reducing the selection pressure. This can be achieved by applying insecticides intermittently at sufficiently high concentrations that are lethal to the majority of insects in the population. Susceptible

individuals will immigrate into the treated population to dilute the survivors. At the next treatment, the majority of individuals in the population after immigration of susceptible flies and successful mating will be susceptible or heterozygous for resistance, and will be killed by the next high dose. This cycle can continue without appreciable increase in resistance. If insecticides are alternated, however, flies that survive the first insecticide will be killed by the high dose of the next insecticide in the alternation irrespective of whether effective immigration of susceptible flies took place. The number of insecticide treatments (selection pressure) in a season can be reduced farther by incorporating other non-insecticidal control methods, e.g. biological control, in the horn fly management programmes.

Insecticides can be applied intermittently and at high doses in sprays (Sheppard 1987) or in dust bags and backrubbers (Harvey and Brethour 1986) or in oilers. Most cattle producers in Manitoba have abandoned these devices (Chapter III, Section I) in favour of insecticide-impregnated ear tags. However, unless there is a breakthrough in the tag delivery system so that insecticides are released intermittently, then probably the only option available to producers is to go back to backrubbers, dust bags and oilers. These devices can release insecticide intermittently if they are charged with limited quantities

of insecticide to last for a few days. They can then be recharged with a different insecticide. Furthermore, these devices will enable producers to consider treatment thresholds which was not possible with ear tags.

CHAPTER V

SUMMARY AND CONCLUSIONS

Pyrethroid-impregnated ear tags were rapidly adopted by beef and dairy producers in Manitoba, although the latter group was slow in adopting tags. In 1987, most of the producers (59% of respondents who apply tags) were applying tags at the rate recommended for horn fly control; 37% were applying less than one tag per animal. Fifty-six per cent of producers had been using ear tags since 1983. Over 95% of the producers were satisfied with the fly control ear tags were affording.

The reports of satisfactory horn fly control made by producers were confirmed in a field survey for resistance to pyrethroids among horn fly populations in Manitoba in 1987. Low levels of resistance, ranging from 0.1- to 5.5-fold for fenvalerate and 0.6-14.0-fold for permethrin, were observed. Tolerant flies were collected from 3 herds, and resistance factors were less than 6-fold to both fenvalerate and permethrin except for one population at Carman that was 14-fold resistant to permethrin. Flies were considered resistant to fenvalerate and permethrin when the resistance factors were equal to or greater than 3-fold. In 1988, resistance factors ranged from 0.03- to 38.0-fold for fenvalerate and 0.8- to over 100-fold for permethrin. Six resistant horn fly populations were identified. In 1989,

resistance factors ranged from 1.0- to 62-fold for fenvalerate and 0.8- to over 100-fold for permethrin. Ten herds had resistant horn flies. The number of resistant populations and intensity of resistance increased from 1987 to 1989. Resistant horn fly populations were interspersed among susceptible ones. Appearance of highly resistant horn fly populations on herds in the Eastern region in 1989 led to the hypothesis that resistant flies were dispersing from the south into Manitoba.

Residues of fenvalerate and permethrin on cattle hair deposited by two tags per animal and one tag for every other animal varied both in space and time. Cattle with two tags per animal had significantly higher residues ($P < 0.05$) than cattle with one tag for every other animal for only one month after tag application at most. There were no differences ($P > 0.05$) in residues on the sampled regions of cattle with or without a tag in the same herd. Residues on the head and neck region of cattle with two tags per animal were greater ($P < 0.05$) than residues on the body or rump during the first 28 days following tagging but there were no differences ($P > 0.05$) in residue levels on the body and rump. Residues determined on hair on days 14 and 84 following tag application declined by 80 to 86% on the head, 73 to 78% on the body and 36 to 84% on the rump. Rainfall reduced residues on hair. The isomeric composition of fenvalerate and permethrin was consistent throughout the season. The

ratio of %SR,RS and SS,RR fenvalerate varied between 51-61% and 39-49%; %cis and trans permethrin varied between 33-39% and 61-67% during the study, respectively. There were no differences ($P>0.05$) in the number of flies observed on cattle that had two tags per animal compared to one tag per two animals.

Pyrethroid-impregnated ear tags selected for resistance rapidly. Resistance factors increased from 2.3- to 17.4-fold for fenvalerate and 1.6- to 8.2-fold for permethrin in one season. Resistance levels declined between fall and spring presumably due to increased survival of flies heterozygous for resistance near the end of the season. Ear tags eliminated susceptible flies from treated populations throughout the season as evidenced by low mortality at discrimination doses of fenvalerate and permethrin. Persistent but declining residues, and elimination of susceptible flies from treated populations appear to be the major contributing factors to rapid development of pyrethroid resistance in horn fly populations. Tagging rates do not appear to play a major role in the development of resistance.

Pyrethroid resistance in two horn fly populations was stable through one season in the absence of selection pressure. Fenvalerate and permethrin ear tags still cannot control pyrethroid-resistant horn flies after one year abstinence from tag use.

Tags containing diazinon alone or in combination with cypermethrin and λ -cyhalothrin alone or in combination with piperonyl butoxide were effective in controlling pyrethroid-resistant horn flies but they still have the same shortcomings as any ear tag formulation. Resistance to them will soon appear.

The most appropriate and long term chemical strategy to manage resistance in horn flies is to alternate insecticides within a season and apply them intermittently. This cannot be achieved with the current ear tag formulations that release insecticide continuously for the whole season. Backrubbers, oilers and dust bags can apply insecticide intermittently if they are charged with limited quantities of insecticide to last a few days. Alternation of insecticide should cover a large area to avoid exposing generations of horn flies to the same insecticide continuously.

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APPENDIX A: Pyrethroid-impregnated Ear Tag Use and Horn Fly
Control Questionnaire.

INSTRUCTIONS :

- 1). Answer the questions by filling or ticking (✓) in the appropriate boxes.
- 2). Return the completed questionnaire to the address on the prepaid envelope as soon as possible.

=====

1. Name of farmer: _____

2. Address: _____

Telephone: _____

3. Type of enterprise:

a). Beef _____

b). Dairy _____

c). Mixed _____

4. If beef, what is the type of operation?

a). Cow-calf pasture system _____

b). Growing finishing using Range and Feedlot _____

c). Growing finishing in Feedlot _____

5. If dairy, what is the type of operation?

a). All year confinement operation _____

b). Winter confinement and summer drylot _____

c). Winter confinement and summer pastures _____

6. Are you using ear tags for fly control?

a). Yes _____

b). No _____

7. If Yes:

a). Specify number of years used _____

b). Type of ear tag:

i). Bovaid _____

ii). Atroban _____

iii). Debantic _____

c). Tagging rate:

i). Less than one tag per animal _____

ii). One tag per animal _____

iii). Two tags per animal _____

8. If No, tick method in use:

a). Spray _____

b). Backrubbers _____

c). Dustbags _____

d). Oilers _____

e). Other _____ Specify _____

f). None _____

Chemical(s) being used: _____

9. How effective is the control method against flies?

a). Very good _____

b). Fair _____

c). Poor _____

10. Do you have handling facilities?

a). Yes _____

b). No _____

11. Are you willing to co-operate with a research team?

a). Yes _____

b). No _____

Thank you very much for your co-operation