THE EFFECTS OF BITING FLIES ON THE WEIGHT GAIN AND BEHAVIOUR OF DAIRY HEIFERS

by

Wendy Elizabeth Ralley

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This thesis is dedicated

to my Mom and Dad

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ABSTRACT

Horse flies and mosquitoes were monitored at the Seven Sisters Grassland Project, Manitoba, and their effects on weight gains and behaviour of pastured dairy heifers observed during the summer of 1983 and 1984. The pasture (timothy and alfalfa) was surrounded by a low-lying balsam-fir fen with poor drainage and supported the breeding of mosquitoes and horse flies. In 1983, thirty-four heifers of approximately the same age and weight, and forty in 1984 were equally divided into 2 herds; one received a biweekly, 1.0 gm a.i. whole-body-spray of cypermethrin and the other was a control herd. Animals were weighted biweekly, 4 times in 1983 and 3 times in 1984. Average daily gains of the treated and control herds were 0.82 ± 0.08 kg and 0.82 ± 0.07 kg (1983), respectively, and 1.03 ± 0.13 and 0.90 ±0.14 kg (1984), respectively. There were no significant treatment differences (P=.05) in either 1983 or 1984, however, significant differences occurred in the variation of weight gains due to spray periods, and the interactions of treatment by period, year by period, treatment by producer within year and producer within year. Cypermethrin killed approximately 100% of mosquitoes up to 5 days post-treatment, and horse flies up to 13 days post-treatment when the flies were exposed for 5 minutes on the animal in bioassay experiments.

Horse flies became active at approximately 0830 hr, then peaked from between 1100 hr to 1530 hr and they remained active until approximately 2000 hr. They were the most bothersome flies to the heifers. Horse flies and mosquitoes caused an increase in individual comfort responses such as head tosses, foot stomps, ear flicks and tail switches. There were no significant treatment differences of these comfort responses. Formation of grazing lines and bunching were the most noticeable changes in response to biting flies. Daily counts of the individual behaviour responses were collected during the peak of daily tabanid activities. These data were divided into 4 separate groups with different combinations of treatment (control and treated) and time periods (beginning of the fly season, and the end of the fly season). Daily count data were analyzed using canonical discriminant analysis which identified in significant treatment differences at the end of the fly season when populations of horse flies peaked.

INTRODUCTION

Biting flies are a seasonal problem for both man and livestock in North America. Many species of biting flies which are pests of livestock in Manitoba breed in vast expanses of undeveloped and pasture land. Blood-sucking flies affect physiological and behavioural processes in cattle through blood-loss, irritation and disease transmission (Krinsky 1976; Fredeen 1977a; Harvey and Launchbaugh 1982; Haufe and Weintraub 1985). Irritation by flies can alter grazing patterns (Schmidtmann and Valla 1982), disrupt digestion and cause general worry to the animals. The harassment of cattle is highly visible and difficult to measure and qualify, but reduced weight gains and milk yeilds may result.

Thirty-four percent of all farmland in Manitoba is pasture (Census Canada, 1983) which amounts to approximately 5.2 million hectares (Eilers et al., 1977). Most of this pastureland is marginal class 3 and 4 soil type. The eastern region of Manitoba is characterized by organic soil with poor surface drainage (Eilers et al., 1977). The soils are commonly called peaty, muck or bog soils and are saturated with water for prolonged periods. Because of its poor quality and high risk for growing cash crops, this land is used for forage production.

In Manitoba, the pasture period for most cattle operations begins in mid to late May, and usually ends in October. The aim of management in cow-calf operations is to have the calves and dams on pasture as early as possible in the spring, until the latest possible time in the fall, with maximum weight gains of the calves from the available forage without damaging existing forage reserves. The average stocking rate in Manitoba is approximately 2.5 acres/ cow-calf, but this depends upon the forage quality and quantity. In highly productive dairy operations, lactating cows require daily grazing on fresh pasture to maximize production (Clark 1977). Any factor that reduces weight-gain or milk-yield is money lost to the producer, and one of these important factors is the attack of biting flies.

This study was conducted at the Seven Sisters Grassland Project (SSGP), located in eastern Manitoba. The pastures at SSGP are surrounded, in part, by a low lying, balsam-fir fen where water collects in depressions from spring snow-melt and after heavy, summer rains. Directly east of the project site (< 1km) lies a dominant, sandy/gravelly ridge in which the soils have low water retention. The SSGP was chosen as a study-site because of a history of on-going research in livestock entomology with the Department of Entomology, University of Manitoba and the Manitoba Department of Agriculture. The most important biting flies

in this region are the Tabanidae and Culicidae. The producers in this are aware of the effects of biting flies blood-feeding on cattle. Biting fly research was initiated here in 1980 because of concern expressed by producers and livestock specialists over the large numbers of biting flies. A herd of cattle relatively uniform in age, weight and breed, as well as necessary equipment, were available at this site.

The null hypothesis in this study was that no significant difference in weight gain and behaviour would be found between the treated and untreated groups of animals. The objectives of this study were:

1. to monitor the abundance and species of Tabanidae and Culicidae, and their relative intensity of attack,
2. to compare weight gain performance between a group of animals treated with cypermethrin and an untreated group of animals,
3. to observe and record differences in behavioural responses to biting fly attack between animals treated with cypermethrin and untreated animals, and
4. to determine the duration of activity of cypermethrin as applied to the animals, using mosquitoes and horse flies in a bioassay.

REVIEW OF THE PERTINENT LITERATURE

The review of the literature is divided into three sections. The first section includes the nuisance flies found on cattle in Manitoba. A general discussion of blood-loss, selection of the host, dispersal, and landing patterns on their hosts is reviewed. The second section is a review of the literature on animal behaviour resulting from attacks by biting flies. The last section is a discussion of the methods of control for biting flies. The literature on this topic is vast and, as a result, only a brief overview of the historical development of control and the modern methods of control are included.

BITING FLIES

There are approximately 140 species of blood-sucking flies in Manitoba, belonging to Culicidae (Wood et al., 1979), Ceratopogonidae, Simuliidae (R.W. Crosskey pers. communication), Tabanidae (H.J. Teskey 1969, unpublished) and Muscidae. Most females of mosquitoes, biting midges, black flies, horse flies, horn flies and stable flies are anautogenous, and both sexes of horn flies, and stable flies feed on blood. In Canada, biting-flies have been implicated in transmitting disease-causing organisms to cattle, either biologically or mechanically (e.g.,anaplasmosis, tularemia) (Greenberg 1971; Krinsky 1976; Laird et al., 1982).

There are no estimates of economic losses to livestock production in Manitoba due to biting flies. It has been suggested that losses in production must be defined by loss per parasite (Sutherst 1983). This could be difficult, considering that a variety of biting flies can be feeding on an animal at one time. However, some estimates of blood-loss have been made by measuring the size of blood meals of mosquitoes (Gwadz 1969), tabanids (Hollander and Wright 1980), black flies (Bennett 1963), biting midges (Tempelis and Nelson 1971), and muscids (Anderson and Tempelis 1970). The potential blood-loss to an animal can then be calculated depending upon the number of flies which feed on the host. Gooding (1972) in his comprehensive review of haematophagous insects, listed the size of blood-meals for some species of culicids (range of 0.001 - 25mg); ceratopogonids (range of 0.1 - 0.23mg); simuliids (range of 0.001 - 0.003mg); tabanids (range of 0.002 - 344mg); muscids (Stomoxys sp. range 6.9 - 16.43mg). Larger flies have a greater capacity for blood, thus, when these flies are abundant, large amounts of blood can be taken from mammalian hosts. For example, the total amount of blood-loss caused by tabanids can range from 75 cc to 352 cc per animal per day (Tashiro and Schwardt 1949, 1953; Miller 1951; Hollander and Wright 1980), depending upon the species of tabanid involved (Hansens 1979). Blood-loss is a measurement of the direct effect of biting-flies on livestock, but this does not include losses due to the irritation caused by them.

Some generalizations can be made for host selection and successful feeding by blood-sucking flies. Much of the information about host selection and feeding has been generated through research dealing with reservoirs and alternate hosts for pathogens and economic assessment of damage to the livestock industry (Steelman 1976; Haufe and Weintraub 1985). Certain generalizations can be made on the mechanisms for host-selection and conditions for successful host-finding. Meteorological factors affect the general activities of some insects. Favourable climatic conditions for successful host-finding include high temperature, low wind speed and turbulance, low barometric pressure, and changing light intensity (Hocking 1971). Crepuscular feeders (some culicids and ceratopogonids) rely on certain cues from the host such as odour, carbon dioxide, and body temperature for successful host-finding (Gillett 1979; Gillies 1980). Diurnal feeders, including tabanids, muscids and some simuliids, to a large extent, are stimulated visually to a host (Hocking 1971). Fredeen (1969) and Shipp (1985) found that large numbers of simuliids were attracted to 'cow- silhouette' traps. Bradbury and Bennett (1974b) stated that long range attraction of simuliids was initiated by odour from the host. Following the concentation gradient of carbon dioxide to the host, black flies were stimulated visually when within 1.8m of the host (Bradbury and Bennett 1974b). Stimuli such as odour and other chemicals probably are involved in probing activities. Vision is important in

host-finding in tabanids. Thorsteinson (1958) and Bracken et al., (1962) found that certain tabanids were attracted to highly reflective glossy spheres. Carbon dioxide is a powerful attractant for tabanids (Anderson et al., 1974; Vale and Phelps 1974) and the Manitoba Horse Fly Trap supplemented with carbon dioxide was far more attractive to the flies than were those without (J. F. Burger University of New Hampshire, pers. communication). The discrimination of colour and shape by black flies and tabanids has been noted by Bradbury and Bennett (1974a) and Brown and Bennett (1980). The shapes of the silhouette traps were seemingly unimportant to simuliids and tabanids, but orientation of tabanids was directed more towards blue, and red traps, and simuliids were attracted to black, red, and blue (Brown and Bennett 1980).

Wind can limit host-location by certain culicids and ceratopogonids; however, it can also aid in the dispersal of some of these flies. The dispersal of mosquitoes is influenced by wind, longevity of the species, and the presence of suitable hosts (Hocking 1953). At wind speeds above 5.45 km/h, mosquitoes either settled down on vegetation or were carried down wind (Clements 1963). The intrinsic flight range of three species of Aedes was about 50 km in still air, though, this potential for long range flight does not include all aspects of climate (Hocking 1953). Most species of ceratopogonids fly short distances

from their breeding sites (within 500 m of the emergence site); however, dispersal is greatly affected by the wind (Kettle 1977).

The landing patterns and site selection of different biting flies on their hosts have been reported by various authors (Smith et al., 1970; Anderson 1973; Mullens and Gerhardt 1979; Schmidtmann et al., 1980; Townley et al., 1984). Host partitioning is most evident with tabanids. Philip (1931) and Blickle (1955) observed tendencies of some tabanids to seek certain areas of the host. These early reports were supported by the work of Smith et al. (1970), Mullens and Gerhardt (1979), and Magnarelli and Anderson (1980). Landing patterns of some tabanids were stratified up the torso of cattle (Mullens and Gerhardt 1979). Larger species of tabanids, in significantly higher numbers, landed on the upper torso which had a heavy hair coat. There was a positive correlation between the length of the hair coat at the landing site and the length of the mouthparts of the species of tabanids (Mullens and Gerhardt 1979). Magnarelli and Anderson (1980) observed similar preferences by tabanids for certain body regions of the host. Large species of Tabanus and Hybomitra were attracted to the sides and backs of cattle. Smith et al. (1970) found that Chrysops were almost always found attacking the head, neck and antlers of white-tailed deer. Selection by black flies of particular areas of the host has been shown by Bennett et al. (1972),

Peschken and Thorsteinson (1965), and Wenk (1981). Simuliids orient to specific parts of wooden dummies of horses with different species attracted to protruding parts and others to the underparts (Wenk 1981). Culicoides spp. were attracted in the greatest numbers to the belly region of calves, sheep, and ponies (Schmidtmann et al., 1980), but to the mane and lower part of the legs of horses.

The reasons for the selection of specific feeding sites on the host are probably a result of subtle chemical and physiological cues including carbon dioxide, body heat, skin odours, and length of hair (Magnarelli the Anderson 1980). The correlation between the length of the mouthparts in certain tabanids (Mullens and Gerhardt 1979) could explain the distribution of larger flies to the areas of the body where the hair was longer. The hairy coat could be a physical barrier to many species. The larger species of tabanids should also be capable of feeding in areas where the skin is easily accessible and therefore hair length alone would not explain selectivity of feeding sites. The selection of feeding sites might also tend to reduce interspecific competition for these sites (Magnarelli and Anderson 1980), since a variety of blood feeding flies could be present at one time.

THE EFFECTS OF BITING FLIES ON THE BEHAVIOUR OF LARGE MAMMALS

There are relatively few quantitative studies of the behaviour of large mammals as a result of interactions with blood-sucking flies flies and large mammals. Biting flies frequently affect animal behaviour and subsequently, the effects of irritation on livestock growth and production are evident (Schmidtmann 1985; Harvey and Launchbaugh 1982; Schmidtmann and Valla 1982; Breev 1950). Responses of domestic and wild mammals to biting flies are reflected in 1) group behaviour and 2) individual behaviour.

Flies have their most noticeable effects on aggregation and movements in herding species. Migrating herds of caribou, for example, formed tightly bunched groups when harassed by culicids and simuliids during mid summer (Hemming 1971). Animals in herds seek temporary relief from the flies by forming compact groups on wind-swept edges or on permanent patches of snow. Downes (1984) found that caribou utilized the microhabitats of ridgetops and snowpatches to avoid mosquitoes. Caribou reduced their harassment by mosquitoes by increasing their altitude of where they graze. In Scotland, red deer (Cervus elaphus L.) were found in exposed, windy areas in the highlands during the peak of Tabanus montanus (Meig.) and Haematopota pluvialis L. activity (Darling 1937). Animals are forced to graze on suboptimal range as a consequence. Horses (Hughes

et al., 1981) and ponies (Tyler 1972) stopped grazing during periods when the numbers of biting flies were high. They stood side-by-side, aligned head-to-tail, to take advantage of a neighbour's switching tail (Hughes et al., 1981). During the day cattle and horses moved onto bare ground when tabanid populations were high (Duncan and Cowtan 1980). This form of habitat selection probably reduced the number of attacking tabanids (Duncan and Cowtan 1980). Similarly, Berezanski (1986) observed wood bison in open habitats during the peak of tabanid populations.

Group size in horses affected the number of flies on an individual horse as well as the number of bites the horse received (Duncan and Vigne 1979). More flies were observed on the individuals in small groups than in large groups. An increase in group size or increased aggregation under fly attack was probably a result of each animal reducing its own suseptibility to attack by putting itself between other animals in the group (Duncan and Vigne 1979).

During severe attacks, animals frequently run from concentrations of biting flies. The distress of one animal can stimulate similar behaviour in others; when one runs, they all run. Such was the case with elk when attacked by tabanids (Collins and Urness 1982). Grazing time for the animals was reduced during the peak of feeding by the flies, but elk compensated by grazing during the cooler parts of the day and night (Collins and Urness 1982).

Individual behavioural responses or comfort movements, such as tail switching, head tossing and skin shivering, increase in frequency with increased attack by biting flies. Behavioural responses of grazing cattle to the attacks of tabanids and stable flies resulted in an increase in the frequency of tail and leg movements (Okumura 1977). Horn flies (Haematobia irritans (L.)) caused untreated animals to spend more time resting and less time grazing during the day (Harvey and Launchbaugh 1982). During the night, the animals spent more time grazing and less time resting. Exposed to the same number of flies, red deer attracted fewer biting flies when lying down than when standing (Espmark and Langvatn 1979). During severe harassment by flies, the deer spent over 70% of their time lying down during the day as compared to 33% of their time in the absence of biting flies. Diurnal feeding by wood bison decreased during June and July, when tabanid populations were greatest (Berezanskie 1986). Diurnal feeding increased in August, and this coincided with the decrease in tabanid activity.

CONTROL OF BITING FLIES

Four categories of biting fly control are usually discussed:

1. application of insecticides over wide areas for control of adults;
2. applications of insecticides to breeding sites for the control of larvae;
3. direct applications of insecticides or repellents in the form of dusts, oils, sprays, or from sustained-release systems to livestock;
4. manipulation of the environment.

The practicality of a given method depends largely upon the biology of the target species. Mosquito larvae, for example, live in stagnant pools and are relatively easy to kill if the breeding sites are accessible. Tabanid larvae, however, burrow in mud or moss and are virtually impossible to control with insecticides.

Early methods of chemical control of adult culicids and tabanids involved aerial application of chlorinated hydrocarbon insecticides, usually DDT, but with limited control (Howell et al., 1949; Gerry 1949; Blanton and Husman 1950; Brown and Morrison 1955). Since DDT was banned in Canada in 1970 along with most other chlorinated hydrocarbon insecticides, organophosphates and carbamates have replaced them for the control of biting flies. Adulticiding is not

usually undertaken for the protection of livestock, but rather a health emergency or for controlling flies which are a nuisance to humans (McLintock 1976).

The effectiveness of adulticiding depends upon the target species, the chemical applied, and the method of application. Insecticidal fogs, ultra-low-volume (ULV), sprays, or common aerial spraying can provide some relief from mosquitoes and black flies, but the effects are usually short in duration; i.e., a few days or less (Laird et al., 1982). Hansens (1981) applied resmethrin and permethrin to wooded areas and the edges of cultivated fields. He achieved a 90% reduction in the number of females of Chrysops spp., being trapped up to 8 hours. It was apparent, however, that after 24 hours flies were returning to the sprayed areas.

Insecticidal control of most mosquitoes and black fly larvae is considered to be more effective than treating for the adult stage (Hollebone 1982). The larvae are found in aquatic habitats and these confined areas usually make control possible. Larviciding for horse flies and biting midges, however, is not widely practiced because of habitat related problems (Laird et al., 1982). DDT and other chlorinated hydrocarbon insecticides had previously been applied as larvicides for the control of black flies and mosquitoes (Mitchener 1953; Jamnback 1973). Although these chemicals gave excellent control of the larvae (Twinn and

Peterson 1955), their effect on non-target organisms was severe (Wallace and Hynes 1981) and resistance of the flies to the chemicals began to appear (Jamnback 1973). Methoxychlor is still being used as a larvicide against black flies in Canada.

Bacillus thuringiensis var israelensis (BT H14) is being used extensively for the control of the larvae of mosquitoes (Lacey et al., 1984) and black flies (Colbo and O'Brien 1984; Pistrang and Burger 1984). Applications of BT H14 by Pistrang and Burger (1984) eliminated black fly larvae and reduced biting activity by the adult population near the treatment site. Colbo and O'Brien (1984) obtained similar results and concluded that BT H14 is effective if applied at the appropriate location and at the appropriate time. BT H14 seems to be environmentally safe with minimal effect on non-target organisms (Molloy and Jamnback 1981), however, the cost of control using BT H14 still remains higher than that of most other chemicals (Margalit and Dean 1985).

Large scale applications of adulticides for control of livestock pests are rarely conducted because of the high cost, minimal effectiveness, and potential for environmental impact caused by the chemicals. Biology of the target species could also render this type of control useless. Livestock producers should then provide some kind of protection for the animals against biting flies. Development of insecticide-application equipment, which

allows self-treatment by range animals, as well as sustained release systems, revolutionized fly control on livestock. Handling of the animals by the producer is reduced and sufficient control of some flies can be maintained by repeated treatments. Self-treatment devices, such as dust bags (Hargett and Turner 1958) and back rubbers (Rogoff and Moxon 1952), have been studied in forced-use and free-choice positions with satisfactory results (Kessler and Berndt 1971; Ronald and Wengo 1973). Plasticized ear tags impregnated with insecticides were probably the most important advancement in sustained-release systems. Ear tags allow for control of insect pests in close association with their hosts, with a single application over the entire season (e.g., lice, horn flies). The costs of repeated treatments and maintenance are virtually eliminated. Excellent control of horn flies has been achieved using ear tags impregnated with a variety of organophosphorus (Harvey and Brethour 1970), and synthetic pyrethroid insecticides (Ahrens and Cocke 1979; Sheppard 1980; Williams et al., 1981; Haufe 1982; Harvey et al., 1983; Harvey and Brethour 1983; Burton et al., 1984). Ear tagging of fewer animals, other than at the recommended rate, has led to concern regarding resistance which has already appeared in horn flies in southern U.S.A. Prolonged low dosages of chemicals, which would result from the use of less than 1 ear tag per animal reduces the cost of control for the producer but favours the development of resistance (Sutherst

1983). Sustained-release chemicals (e.g. avermectins) are highly effective as larvicides of horn flies and stable flies (Miller et al., 1981). However, these compounds also reduce the number of other dung-feeding insects, some of which are predators and parasites of Haematobia, Musca and Stomoxys (Sutherst 1983; Schmidt 1983; Drummond 1985).

Although little is known about how insect repellents prevent insects from biting their hosts (Davis, 1985), many types of repellents have been tested for the protection of humans and livestock against biting flies. The most successful has been N,N-diethyl-m-toluamide (DEET). When formulated as an aerosol spray, DEET was a relatively poor repellent against stable flies and tabanids on horses and cattle (Blume et al., 1971). DEET, although well tolerated by humans, when applied to other animals at dosages that provided sufficient protection from biting flies, caused severe skin irritations (Palmer 1969; Blume et al., 1971). Repellency has also been reported for synthetic pyrethroid chemicals applied to livestock as whole-body sprays. Shemanchuk (1981) compared the effectiveness of permethrin, cypermethrin, and resmethrin in preventing black flies from taking a blood meal. Permethrin, at a dosage of 12 mg a.i./Kg of body weight, repelled black flies effectively for 11 days. Cypermethrin at 2mg a.i./Kg repelled black flies for 4 days and resmethrin at 6mg a.i./Kg repelled black flies for 2 days. In laboratory tests and field trials,

permethrin gave cattle and horses satisfactory protection against stable flies, horn flies, and biting midges for 4, 15, and 10 days, respectively (Blackman and Hodson 1977).

Permethrin EC at 2.0% a.i. eliminated all horn flies from a cattle herd for at least 1 week when applied to only 1 animal (Harvey and Brethour 1979). Similarly, permethrin, at 250ml of 0.1% and 500ml 0.1% per animal controlled horn flies for 3 weeks and stable flies for 2 weeks, respectively (Bailie and Morgan 1980). Harris and Oehler (1976) studied the effectiveness of several compounds on horses, under field conditions and permethrin was the most effective (up to 2 weeks at rates of 0.5g a.i./animal). Their results were supported by Bay et al. (1976). Permethrin as an emulsifiable concentrate (EC) (.05% and 0.1% a.i.) and dust (.25%) provided excellent control of tabanids on treated horses and cattle (Bay et al., 1976).

Environmental manipulation of small isolated breeding sites of some biting flies can be effective in reducing adult populations. Habitat destruction for oviposition and larval development has been demonstrated as a method of reducing mosquitoes (Williamson 1949) and horse flies (Anderson and Kneen 1969). Aquatic habitats which produce mosquitoes can be eliminated by the impoundment of water or by altering the water level by drainage (Laird et al., 1982). Oldroyd (1964) suggested controlling Chrysops in isolated ponds by raising the water level to drown the

larvae and pupae in the soil. This method, tested by Anderson and Kneen (1969) in Connecticut, resulted in a substantial reduction in the larvae of Chrysops fuliginosus Weid. The removal of emergent vegetation from small ponds and seepage areas can reduce oviposition sites for tabanids significantly (Pechuman 1981). Easton (1982) speculated that cattle allowed to graze along the borders of ponds would reduce available oviposition sites. The tramping of animals would also decrease the number of mature larvae able to pupate and emerge along the pond margin (Easton 1982). These procedures, however, are not practical for large bodies of water and swamps (Pechuman 1981).

Habitat manipulation has also been suggested for the control of black flies (Jamnback 1973). Any alteration in the pattern of flow of water can eliminate species or result in the substitution of one for another (Laird et al., 1982). In areas where breeding is restricted short, turbulent stretches of an otherwise slow-flowing river, the removal of large boulders, trailing vegetation, and logs could reduce or eliminate breeding sites (Jamnback 1973). The construction of dams could also eliminate black flies by creating a lake in the up-stream area. Black fly populations may be affected by the establishment of macrophytes in the river, causing a change in the black fly species (Fredeen 1977b). A dam which allows for release of water could be used to restrict the rate of flow which is

necessary for larvae to complete their development and thus reduce black fly populations (Jamnback 1973).

The control of most biting flies in Canada is primarily accomplished by insecticidal methods. Canadian recommendations for the control of black flies, mosquitoes, and horn flies involve a variety of insecticides applied at different rates and formulations (Laird et al., 1982). Horse flies, however, are probably the most difficult biting flies to control as either adults or larvae. There is no recommendation, federal or provincial, for the control of horse flies (Manitoba Insect Control Guide 1986). Applications of insecticides to wide areas have been only moderately effective while increasing the danger to non-target organisms (Pechuman 1981). Synthetic pyrethroids applied to livestock as whole-body sprays show some promise, but their effects are short-lived.. Ear-tags, although highly effective for horn fly control, provide little or no protection against horse flies. Pechuman (1981) suggested that the most promising method of controlling horse flies in limited areas could be the use of trapping devices. Large numbers of box traps have been used in Massachusetts to protect beach areas from the large populations of T. nigrovittatus L. (Spence 1971 as cited by Hansens 1979). In New York, canopy traps (modified Manitoba Horse Fly Traps) are used commercially around paddocks where stud horses are kept and where Equine Infectious Anemia is a problem. These

traps do not eliminate the horse fly problem, but in some cases they reduce the populations to tolerable levels. The control of tabanids, however, remains impractical, especially where extensive breeding habitat is available.

THE EFFECT OF BITING FLIES ON THE WEIGHT GAIN OF DAIRY HEIFERS AND A BIOASSAY OF CYPERMETHRIN USING MOSQUITOES AND HORSE FLIES

INTRODUCTION

Blood-sucking flies can cause weight loss and reduced weight gain in pastured cattle (see review by Steelman 1976). Millions of dollars are spent annually in the development and application of control methods as well as monies lost annually in production of beef and dairy (Haufe and Weintraub 1985). Losses in production due to biting flies depend upon the species of fly present and their abundance. Members of the families Tabanidae and Culicidae are the most numerous and pestiferous flies in the area of Manitoba where the current study took place. Although the fly-season is relatively short in Manitoba (2 months), it is during this time that producers expect maximum gains from pastured animals. Their persistence in seeking blood makes horse flies and mosquitoes (especially horse flies) very serious pests of animals.

Many insecticides are registered for the control of mosquito larvae and adults, but adequate and practical control in rural areas is limited because of the size and inaccessibility of the breeding sites. In Manitoba, there are no recommended methods for the control of horse flies.

This study took place at the Seven Sisters Grassland Project (SSGP) which is located in eastern Manitoba. The site was chosen because of the availability of research animals, and populations of biting flies have been monitored

since 1979. The land is poor for the production of cash crops, thus, much of this land is used for the production of forage. The objectives of this study are: 1) to monitor the relative abundance of the important species of biting flies at the SSGP, 2) to measure the weight gains of dairy heifers on pasture at the same location, 3) to assess biting fly control on cattle treated with the synthetic pyrethroid, cypermethrin. would be relatively protected from biting flies.

MATERIALS AND METHODS

Treatments

The dairy heifers used in this study were supplied by producers from various locations in eastern Manitoba (see Appendix A). Forty animals were supplied by 20 producers in 1983; each producer supplied 2 animals. The initial weights and ages of the animals ranged from 212 to 386 kg ($\bar{x}$=280) and 9 to 17 months, respectively. All animals were brought to the site, weighed and tagged on 3-6 June, 1983. During this time, the animals were released on pasture to forage prior to any spraying and this time was referred to as the adjustment period. There were 39 black and white Holsteins and one red Holstein. The animals were divided into two herds on 7 June; one animal from each producer represented in each herd. Each animal in one herd received

a 2L, full-body-spray of 0.1% (2gm a.i.) cypermethrin biweekly. The spray was delivered using a hand-held Bean sprayer (Trojan model No. 2020, Spray gun model No. 57) and was calibrated before each application. All animals were weighed biweekly. This two-week period was referred to as a spray period. In 1983, there were 4 spray periods. The herds were separately grazed on improved, fertilized, orchard and timothy grasses (Dactylis glomerata L. and Phleum pratense L.) in adjacent paddocks of approximately 5 ha. each (Figure 1, Paddock 1, Pastures A and B). A wind row of trees transected each pasture which offered shade to the animals. The herds were alternated on pasture after each spray period to reduce any paddock bias (Figure 2). From 7 to 16 June and again on 25 July, animals in paddock one were fed alfalfa hay in a round bale feeder. Due to the reduction of available forage in both paddocks, and application of malathion for grasshopper control, the project was terminated and the herds combined and pastured in different paddocks after 29 July.

In 1984, the 34 dairy heifers used on this project were supplied by 10 producers (Appendix B). Two Jerseys, 1 Jersey x Brown Swiss and 14 Holsteins were represented in each herd. As in 1983, one red Holstein was present. The initial weights of the animals ranged from 153 to 320 kg ($\bar{x}$=256) and the ages from 8 to 16 months. The animals were brought to the project site on 29 May, and after an

Figure 1. The pasture areas at the Seven Sisters Grassland Project, Seven Sisters, Manitoba. Paddock 1 was the pasture area used in 1983 and paddock 5 in 1984. The location of the Manitoba Horse Fly Trap (MHFT) is indicated by a dot with corresponding number beside it. As well, the location of the New Jersey Light Trap (NJLT) is indicated.

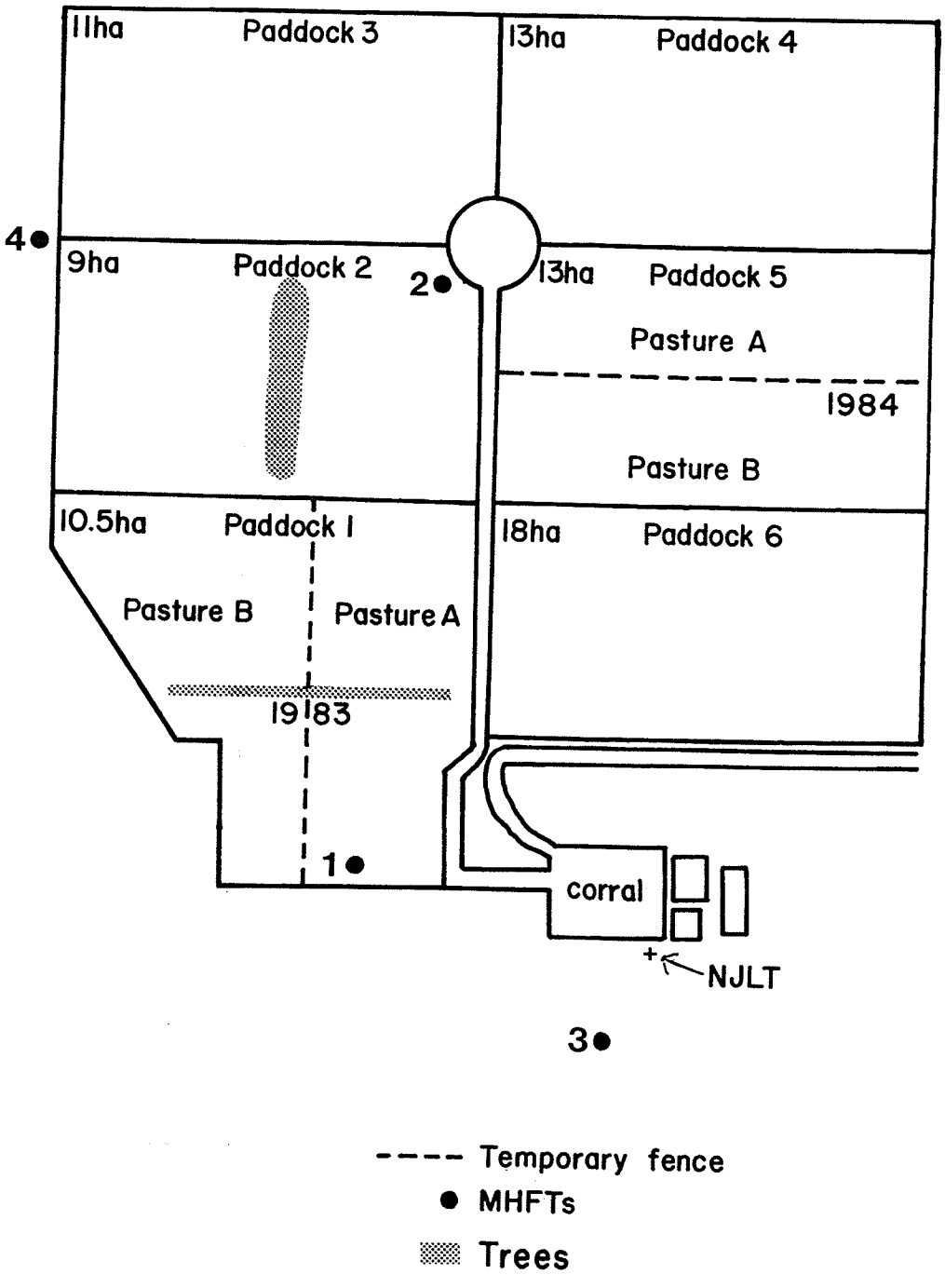
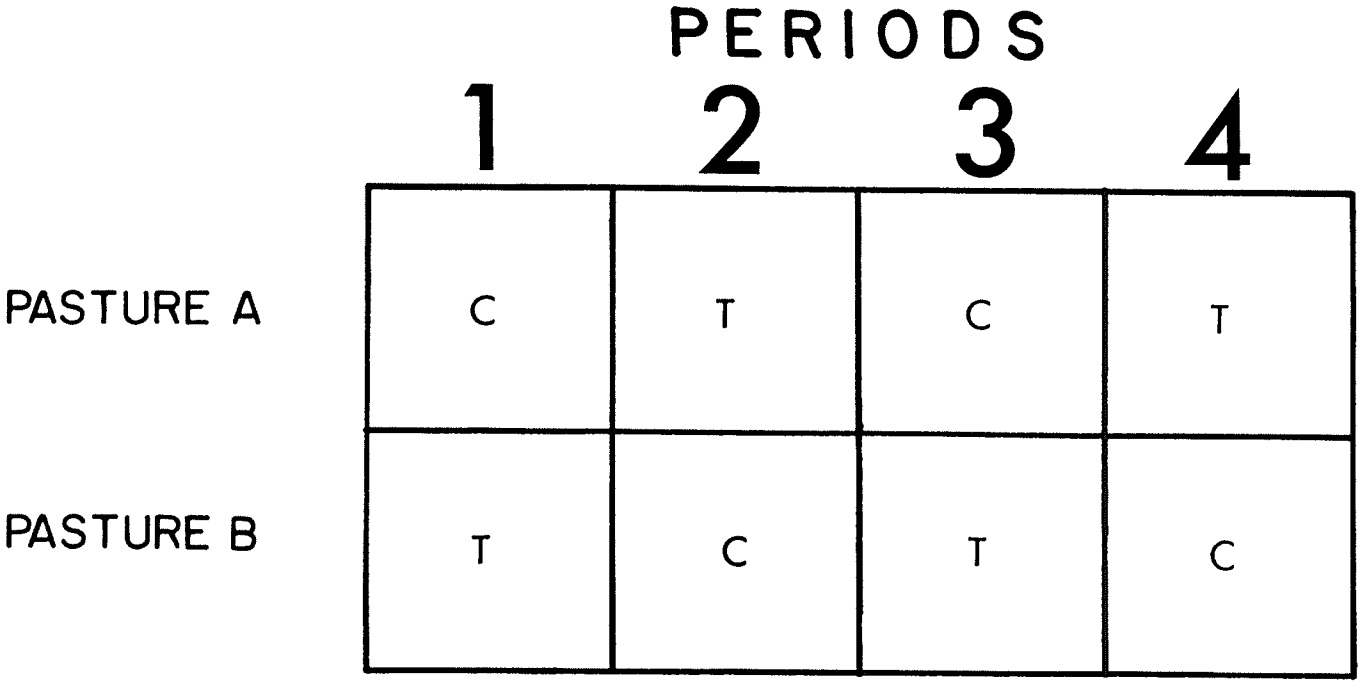

11ha
Paddock 3
13ha
Paddock 4
4
9ha
Paddock 2
2
13ha
Paddock 5
Pasture A
1984
Pasture B
10.5ha
Paddock 1
18ha
Paddock 6
Pasture B
Pasture A
1983
1
corral
NJLT
3
Temporary fence
MHFTs
Trees

Figure 2. A schematic diagram of the pastures and periods for 1983 and 1984. The pastures were adjacent in both years, and the herds switched pastures each period to reduce any bias.



T= treated herd

C= control herd

adjustment period of 15 days on pasture, were divided, weighed and one herd received a 1L full-body-spray of 0.1% (1 gm a.i.) cypermethrin. The rate of application was halved in 1984 because of extensive runoff and chemical loss observed in the 1983 applications. The aperture of the nozzel was also changed from a hard, directed spray to a conical mist to give good body coverage of the spray, with less runoff. Although the animals were sprayed biweekly for 4 periods, final weights in August, 1984 were not taken. As a result, there were 3 spray periods in 1984. The herds were grazed separately on adjacent pastures in paddock 5 (Figure 1) which consisted of orchard and timothy grass, and alfalfa (Medicago sativa L.). Each pasture was approximately 6.5 ha and neither pasture offered any shelter for the animals. The animals were also alternated on pasture by spray period to reduce forage bias.

Biting Flies

Populations of culicids and tabanids were monitored daily using a New Jersey Light Trap (NJLT) and Manitoba Horse Fly Traps (MHFT). Four MHFT (Figure 3) were situated in or near the paddocks during the summer of 1983 (Figure 1). Traps 1 and 2 were operated from 9 June, trap 3 from 16 June and trap 4 from 30 June. Two MHFT were operated at sites 2 and 3 from 10 June, 1984. The design of the traps was altered from 1983 to that as shown in Figure 4. Trap 3 was

supplemented with 4.54 kg of dry ice on 19, 20, 24 July. The trapped tabanids were removed nightly after activity ceased. Mosquitoes were collected twice weekly from 7 June to 18 July, 1983. The NJLT (Figure 5) was operated one half hour before sunset until the following morning. In 1984 mosquitoes were collected daily at the same location as in 1983 (Figure 1). Although populations of black flies and stable flies were not monitored in either year, casual observations were made as to their abundance and effects on the animals. Temperature and rainfall were recorded daily.

Bioassay

In 1984 a bioassay was conducted to determine the efficacy of cypermethrin remaining on the animals over time. Mosquitoes and horse flies were used in assaying the chemical. Flies were retained in cages made from honey containers (6.5 cm deep and the diameter of the top and the bottom is 11.5 and 9.0 cm, respectively) with the bottoms and the tops removed (Figure 6). Each end was covered with netting; 1 mm mesh size for the mosquito cages and 3 mm mesh size for the horse flies cages. The number of mosquitoes (Aedes vexans (Meigen)), used in the bioassays varied from 10 to 20 and the number of horse flies (primarily Hybomitra pechumani Teskey and Thomas) varied from 5 to 10 depending upon availability. Two animals from each herd were chosen for the bioassay based upon their ease of handling in the

Figure 3. The design of the Manitoba Horse Fly Trap during the summer of 1983. The traps were constructed using .025 mm thick polyethylene plastic and aluminum pipe. The 'doughnut' was constructed by connecting four 20.32 cm diameter drainage-pipe-elbows sprayed with high-gloss, black, enamel paint.

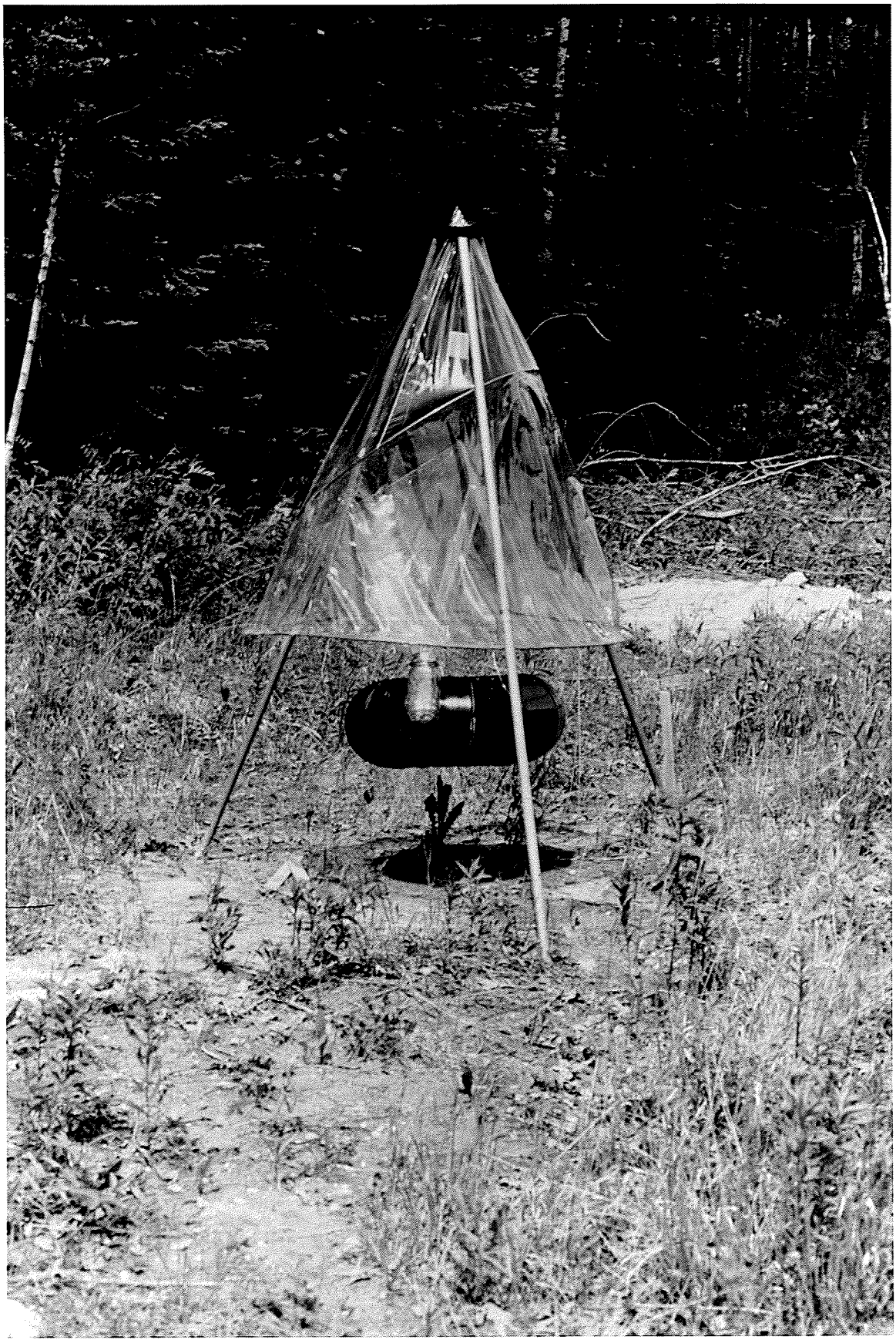


Figure 4. The design of the Manitoba Horse Fly Trap used in 1984. The plastic and aluminum pipes were from the same design as used in 1983. Glossy, black spheres approximately .61 m in diameter (as those used by Thorsteinson et al., 1964) were used for visual attractance. A small container was placed at the apex of the trap, into which the flies were collected.

Figure 5. The New Jersey Light Trap with a 25 watt bulb for monitoring mosquito populations in 1983 and 1984 at the Seven Sisters Grassland Project, Manitoba.

Figure 6. The bioassay cage used for testing horse flies in 1984 at the Seven Sisters Grassland Project, Manitoba.

field and these animals were used for each exposure. Following application of the cypermethrin, a bioassay was conducted on various times post-treatment depending on weather conditions and fly availability (Table 1). The bioassay using horse flies was conducted during the 3rd and 4th spray periods, and a variety of tabanid species taken from the MHFTs that day, were used for the test. The bioassay using mosquitoes was done during the 2nd, 3rd and 4th spray periods when the flies were available in sufficient numbers. The cages were labelled, wrapped in dampened paper towelling, placed in one of two coolers (control, treatment) and taken into the paddocks. The cages were hand-held on each animal for approximately 5 minutes, one on the shoulder and the other midway along the back-line. Moistened cotton batting was placed on each cage, and the cages were put on wet paper towelling. The cages were then placed in an incubator (20 - 25°C) and the mortality was recorded 2 hours after exposure to the animals.

Table 1. Times post-treatment when a bioassay was conducted using mosquitoes (Aedes vexans Meigen) and/or horse flies (spp.) during 1984 at the Seven Sisters Grassland Project, Manitoba. The bioassay was conducted on two animals for each treatment.

Time Post-Treatment	Period 2		Period 3		Period 4	
	Mosq.	H.f.	Mosq.	H.f.	Mosq.	H.f.
1 hour	x		x			
8 hour					x	x
12 hour			x	x		
24 hour	x					
3 day	x					
5 day			x	x	x	x
7 day	x					
12 day			x		x	
13 day	x			x		x

Mosq. = mosquitoes
H. f. = horse flies

RESULTS

1. Biting Flies

Forty-nine species of biting flies were trapped and identified at the SSGP, Manitoba during 1983 and 1984 (Table 2). Of these, eleven species of tabanids, 15 species of culicids and one species of simuliid, which were also observed feeding on the animals. Aedes punctor (Kirby) was the most abundant mosquito collected in the NJLT in 1983 (Figure 7). The population peaked during the 3rd week of June with an average nightly-catch of 153 ± 50 A. punctor in that week. Aedes vexans (Meigen) was the most abundant mosquito trapped in the NJLT in 1984, and the population of this species peaked during the 2nd week of July (2314 ± 889) (Figure 7). Very few flood-water species, (e.g., A. vexans), were trapped in 1983, probably due to the small amount of summer rain (Appendix C) which was not sufficient for the maintenance of pools. Similarly, very few species of the spring Aedes spp. were trapped in 1984, probably due to the scarcity of snow-melt pools. From 6 June until 26 June, 1984, approximately 20.3 cm of rain fell (Appendix C) resulting in the development and emergence of large populations of A. vexans.

Hybomitra spp. were the most abundant tabanids found in this area of Manitoba. The MHFTs were set-up on 8 and 10 June in 1984, however it was noted in both years that horse

Table 2. A list of the species present at the Eastern Grasslands Project, Seven Sisters, Manitoba, during 1983 and 1984.

Culicidae

Aedes abserratus (Felt and Young)*
A. canadensis (Theobald)
A. cinereus Miegen*
A. communis (De Geer)
A. dorsalis (Meigen)*
A. euedes Howard, Dyar, and Knab
A. excrucians (Walker)*
A. fitchii (Felt and Young)*
A. flavescens (Muller)*
A. implicatus Volkeroth*
A. intrudens Dyar*
A. nigromaculis (Ludlow)
A. pionips Dyar
A. provocans (Walker)*
A. punctor (Kirby)*
A. riparius (Dyar and Knab)*
A. spencerii (Theobald)*
A. sticticus (Meigen)
A. stimulans (Walker)*
A. vexans (Meigen)*
Anopheles earlei Vargas
A. walkeri Theobald
Culex restuans Theobald
Culiseta inornata (Williston)*
Mansonia perturbans (Walker)

Simuliidae

Simulium vittatum Zetterstedt*

Muscidae

Stomoxys calcitrans L.*

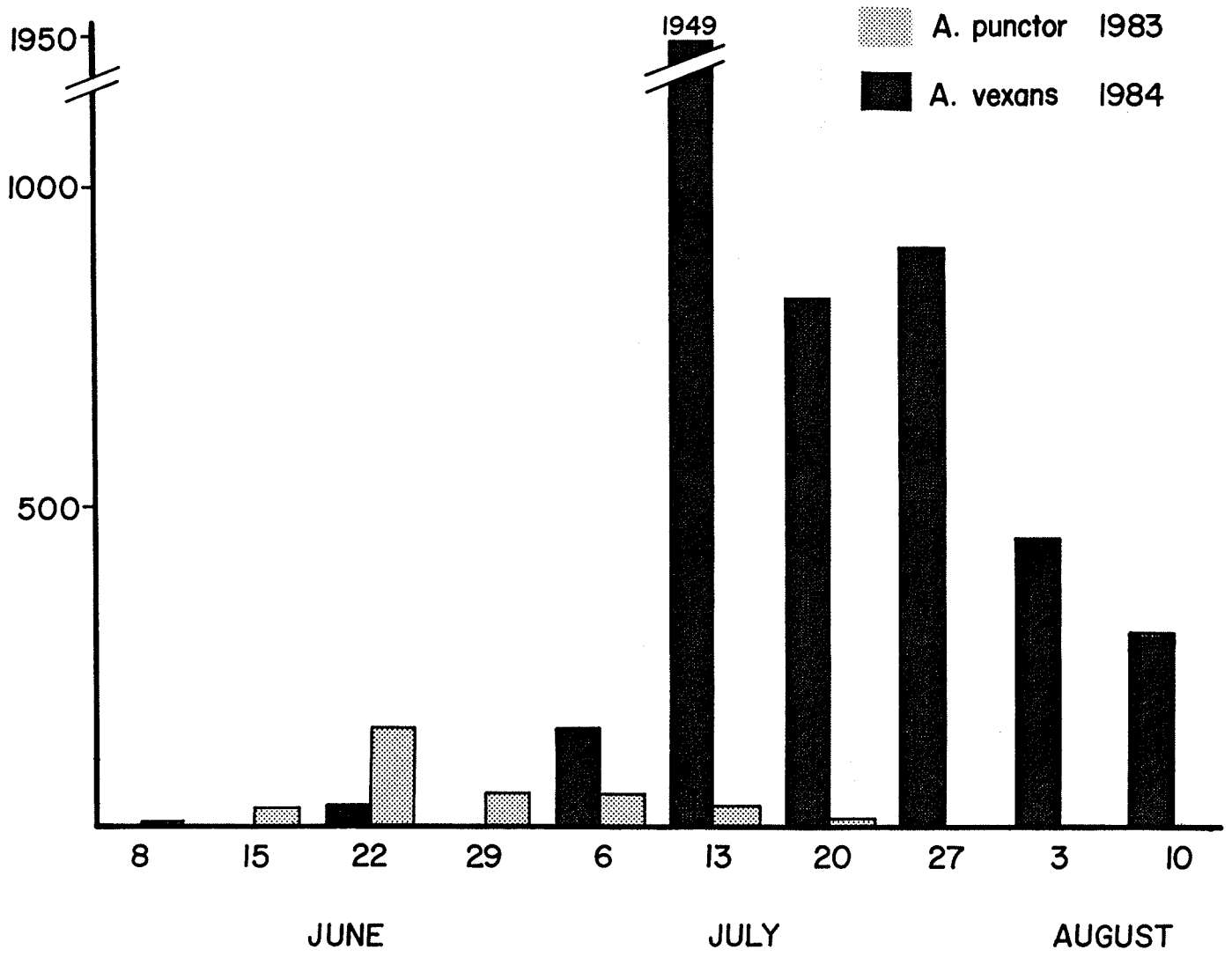
continued....

Table 2. (cont.)

Tabanidae
Chrysops frigidus O.S.
C. excitans Walker
C. indus O.S.
C. mitis O.S.
Haematopota americana Forst
Hybomitra affinis (Kirby)*
H. apardi
H. epistates (O.S.)*
H. frontalis (Walker)*
H. illota (O.S.)*
H. lasiophthalma (Macq.)*
H. lurida (Fallen)*
H. nuda (McD.)*
H. pechumani Teskey and Thomas*
H. trepida (McD.)*
H. zonalis (Kirby)*
Tabanus fulvicallus Philip
T. lineola Fabr.
T. marginalis Fabr.
T. reinwardtii (Weid.)
T. similis (Macq.)*
T. vivax O.S.

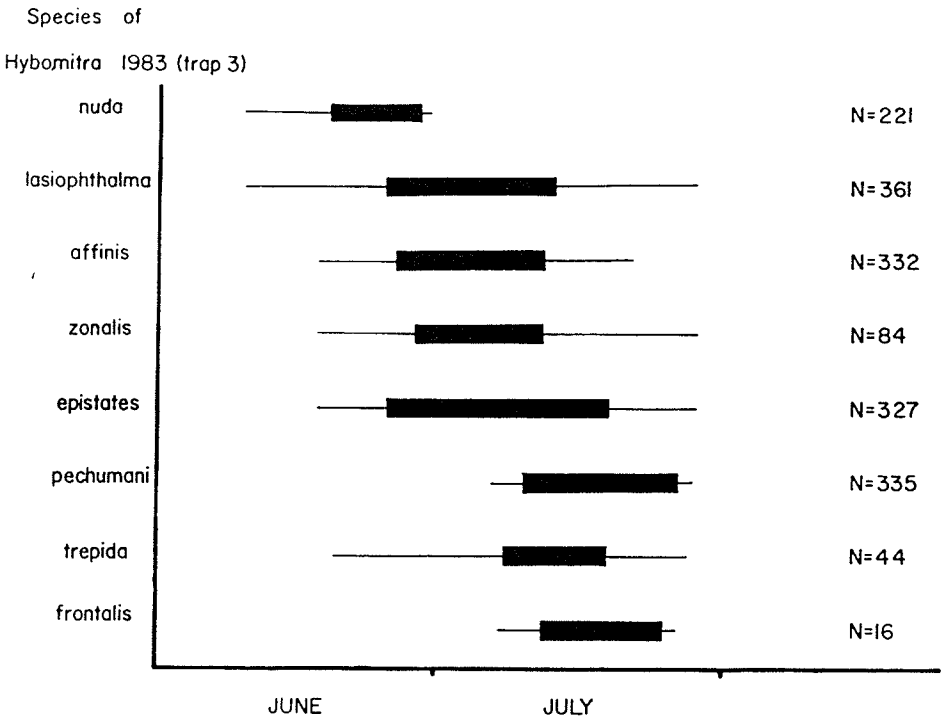
* indicates those species of biting flies which were observed feeding on the cattle at Seven Sisters, Manitoba, during 1983 and 1984.

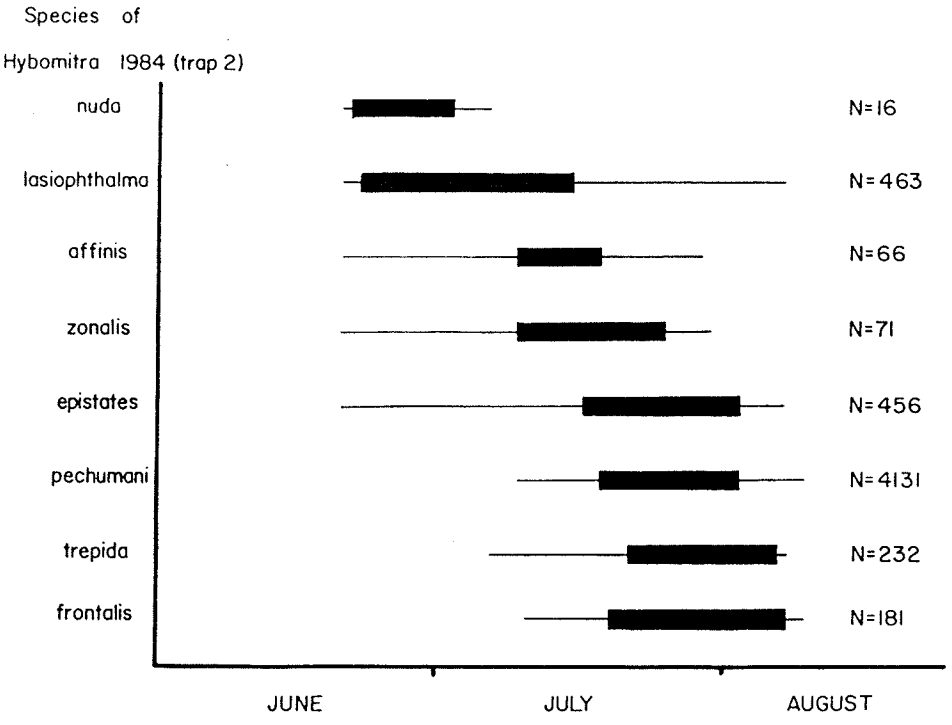
Figure 7. The average weekly totals for Aedes punctor, 1983, and Aedes vexans, 1984, caught in the New Jersey Light Trap at the Seven Sisters Grassland Project, Manitoba.


1950
1000
500
1949
A. punctor 1983
A. vexans 1984
8
15
22
29
6
13
20
27
3
10
JUNE
JULY
AUGUST

flies were present in low numbers before these dates. If a species was caught on one date and not another it was assumed to be present at the study-site and therefore included. There was approximately 1 week difference in emergence of the Hybomitra spp., between 1983 and 1984 (Figure 8). Most species are active from mid-June to early August, and many may be present simultaneously; however, there is a definite emergence pattern. The first species to appear in the season are Hybomitra lurida (Fallen) and H. nuda (McD.). Hybomitra lurida was rare in both years and therefore not included in Figure 8. Hybomitra lurida and H. nuda had virtually disappeared before most of the other species peaked. Hybomitra lasiophthalma (Macq.), however, appeared in the first to last trap-date, and peaked from late June to early July. Although more than 5 times as many H. affinis were trapped in 1983 than in 1984, the populations in both years had virtually disappeared by mid-July. Hybomitra zonalis (Kirby) was not very numerous in either year, but the daily catches in the MHFTs consistently included H. zonalis in low numbers. Hybomitra epistates began to emerge in late June and occurred in all trap catches until the end of the season. Hybomitra pechumani was very numerous, and the emergence of this species was very abrupt. The peak of the population occurred a few days after the first fly was observed on the animals. Hybomitra trepida and H. frontalis peaked late in the season, and their numbers in the traps were consistently low.

Figure 8. A graphic representation of the more common species of Hybomitra at the Seven Sisters Grassland Project, Manitoba during 1983 and 1984. The line represents the first to last date on which these species were trapped in the Manitoba Horse Fly Trap, and the bar represents 75% of the total catch.


Species of
Hybomitra 1983 (trap 3)
nuda
lasiophthalma
affinis
zonalis
epistates
pechumani
trepida
frontalis
N=221
N=361
N=332
N=84
N=327
N=335
N=44
N=16
JUNE
JULY


Species of
Hybomitra 1984 (trap 2)
nuda
lasiophthalma
affinis
zonalis
epistates
pechumani
trepida
frontalis
N=16
N=463
N=66
N=71
N=456
N=4131
N=232
N=181
JUNE
JULY
AUGUST

By far, the most numerous species of horse fly around the animals was Hybomitra pechumani in both 1983 and 1984. Average weekly totals during peak populations of H. pechumani in 1984 were approximately 4 times that of 1983. However, the peak of the 1983 population occurred during the second week of July, and the 1984 peak occurred during the last week of July (Figure 9). Hybomitra pechumani did not appear in the traps before July.

2. Weight Gains

In 1983, the average daily gain (ADG) over 52 days of the treated and control animals was approximately the same, and in 1984 the ADG was 0.13 Kg higher in the treated animals over a period of 57 days (Table 3). Factors other than treatment had to be considered in the analysis of the weights. These data were analysed using a split plot ANOVA (SAS Institution, 1985).

Of the main effects (year, treatment, and period), periods were significantly different (P=.05); i.e., there was a significant difference in weight gains of the animals over time (periods) (Table 4). There were no significant differences of the weights between years, nor between treatments. Significant differences (P=.05) occurred in the variation due to producer within year, the interaction of treatment by producer within year, the interaction of

Figure 9. The average weekly totals for Hybomitra pechumani Teskey and Thomas caught in the Manitoba Horse Fly Traps at the Seven Sisters Grassland Project, Manitoba, during 1983 and 1984.

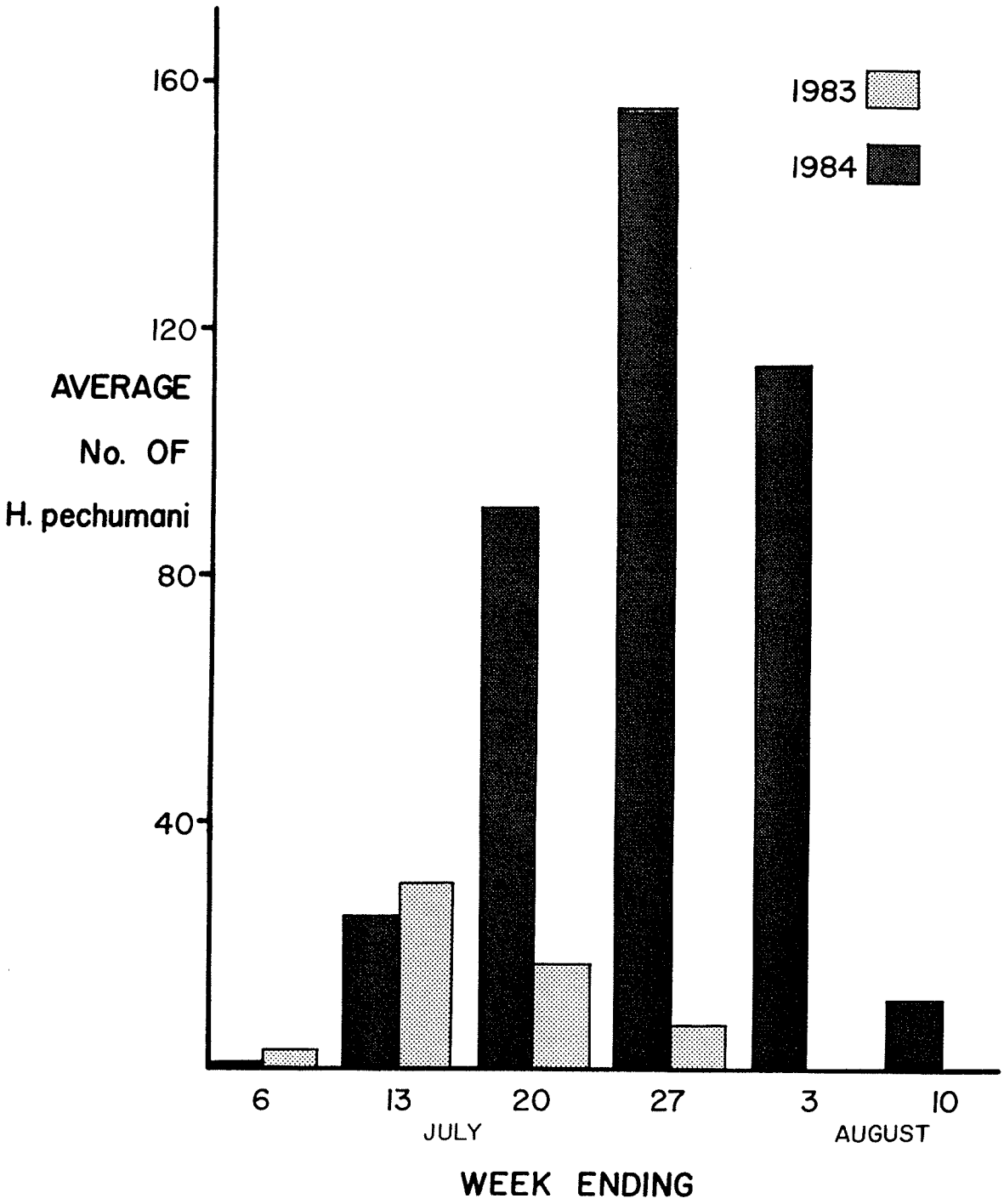

160
120
80
40
AVERAGE
No. OF
H. pechumani
1983
1984
6
13
20
27
3
10
JULY
AUGUST
WEEK ENDING

Table 3. The mean of the total weight and the average daily gain (ADG) in Kg of the treated and control dairy heifers during the 1983 and 1984 season, Seven Sisters Grassland Project, Manitoba.

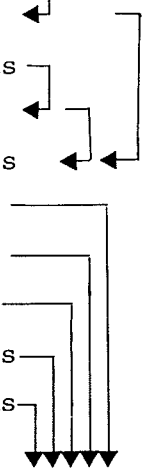
	1984[b]		1983[a]	
	Mean Total Wt. (Kg)	ADG	Mean Total Wt. (Kg)	ADG
Treated N=17	46.9 ± 5.00	0.82 ± 0.08	53.7 ± 6.89 N=19	1.03 ± 0.13
Control N=17	47.0 ± 4.07	0.82 ± 0.07	47.0 ± 7.53 N=20	0.90 ± 0.14

[a] over a period of 52 days

[b] over a period of 57 days

Table 4. The analysis of variance of the weight gain data for sprayed and control animals at SSGP 1983 and 1984. Arrows indicate which error term each source of variation was tested against.

Source of variation	df	F	Prob F	
year	1	.29	.5966	ns
prod(year)	28	8.47	.0001	*
trt	1	1.12	.2999	ns
trt x prod(year)	26	3.53	.0083	*
tag(prod x year x trt)	14	0.11	1.0000	ns
per	3	7.66	.0001	*
trt x per	3	6.53	.0004	*
year x per	3	4.65	.0038	*
year x trt	1	0.50	.4783	ns
year x trt x per	3	1.87	.1337	ns
error b				



* signifigance ($P < 0.05$)
ns not signifigant ($P \geq 0.05$)

treatments within periods, and the interaction of year by periods. Treatment effects on weight gains depends upon the periods or time of the summer, thus it would appear that the differences in weight gains between the treated and control animals are dependent on time. However, since treatments are confounded with pastures, (Figure 10) the variation in weight gain may be due to characteristics of the pastures rather than treatment differences, since treatment effects alone were not significant.

The average daily gain (ADG) of the treated and control animals varied greatly between pasture A and pasture B. In 1983, animals in pasture B during periods 2, 3, and 4, regardless of treatment, gained twice as much as those in pasture A (Figure 10). In 1984 the experiment was conducted in paddock 5 (Figure 1), and animals in pasture A, had higher ADGs than those animals in pasture B (Figure 10). The significance of year by periods interaction may indicate that the weight gains in each period are not comparable between 1983 and 1984.

There were no compensatory gains since there were no significant differences (P=.05) between weight gains from the last spray period, to November of 1983 and 1984.

Figure 10. The average daily gains (ADG) in Kg for the treated and control herds for each spray period. The arrow indicates when the animals were sprayed in 1983 and 1984.

1983

	Period 1	Period 2	Period 3	Period 4
PASTURE A	Treatment ADG=.94	Control ADG=.79	Treatment ADG=-.14	Control ADG=1.24
PASTURE B	Control ADG=.91	Treatment ADG=1.58	Control ADG=.81	Treatment ADG=2.03
	7-21June (14 days)	21June-5July (14 days)	5-19July (14 days)	19-28July (10 days)

Treatment N=19
Control N=20

1984

	Adjustment Period	Period 1	Period 2	Period 3
PASTURE B	Treatment ADG=.52	Control ADG=1.13	Treatment ADG=.57	Control ADG=.59
PASTURE A	Control ADG=.68	Treatment ADG=1.26	Control ADG=.89	Treatment ADG=.99
	29May-13June (15 days)	13-27June (14 days)	27June-12July (15 days)	12-25July (13 days)

Treatment N=17
Control N=17

3. Bioassay

The results of the bioassay are not conclusive since very little replication could be done and thus no statistical analysis of these data was conducted. Virtually 100% of the mosquitoes were killed up until day 5 post-treatment, and 100% of the horse flies were killed up until day 13 post-treatment (Figure 11 and 12). Mosquitoes did not readily feed on the animals when the cages were placed on the coat. As such, the cages had to be agitated to force the mosquitoes down onto the hair and thus exposing them to any chemical which may have been on the hair. Some mosquitoes may have been injured, increasing the percentage of mortality of the controls. Cages containing horse flies were not agitated since they attempted to blood-feed by probing through the hair. This might explain why the mortality of the tabanids was almost 100% at 13 day post-treatment. By attempting to blood feed, the tabanids would have been exposed to any cypermethrin present for a longer and a more consistent time period. Although the cages were held on the animals for 5 minutes, direct exposure to cypermethrin on the hair coat for mosquitoes was less than for tabanids, simply because they did not attempt to feed.

Figure 11. The percent mortality of mosquitoes (Aedes vexans) used in the bioassay for spray periods 2, 3, and 4 at the Seven Sisters Grassland Project, Manitoba, 1984. Lines labelled T and C represent treated and control, respectively.

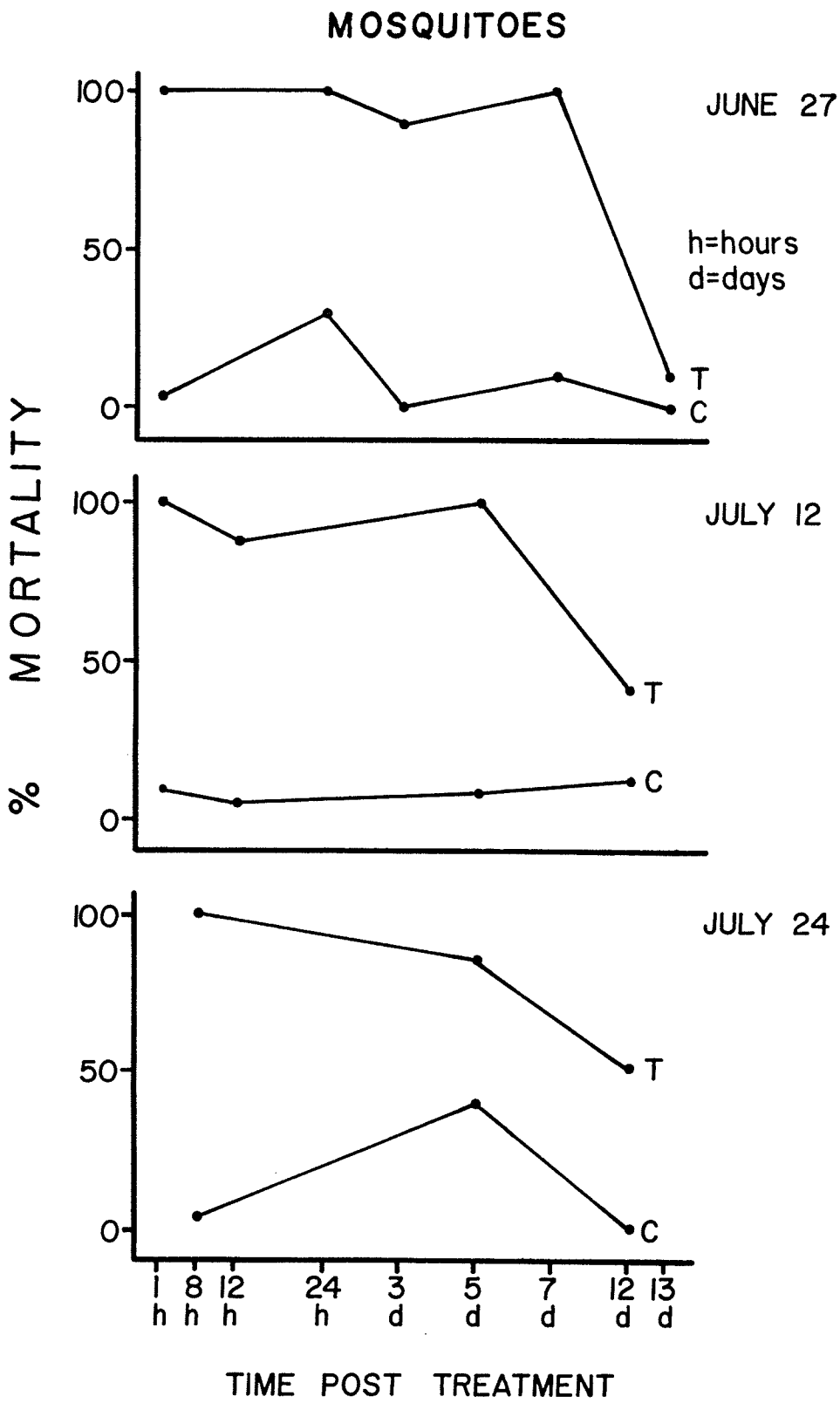
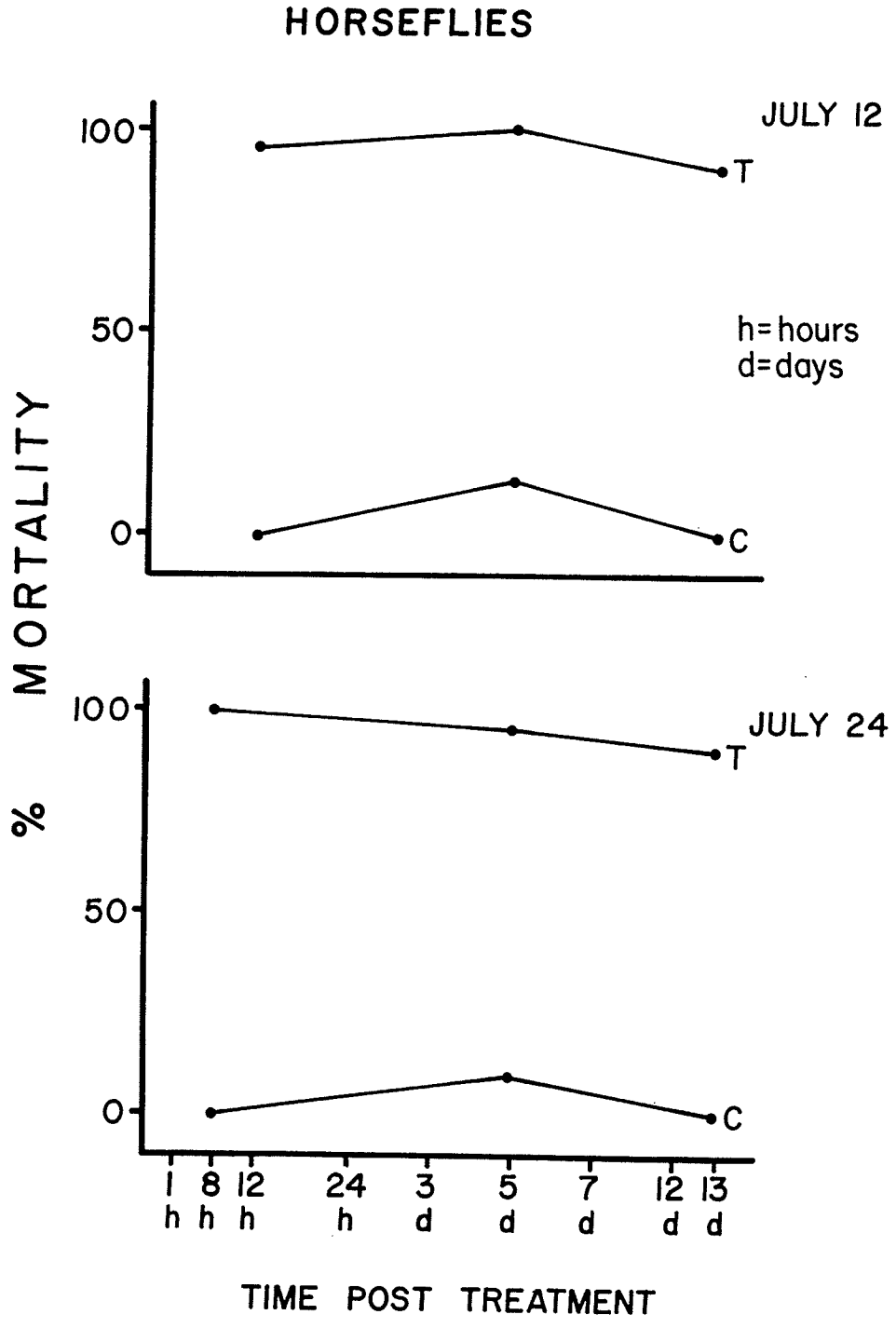

MOSQUITOES
JUNE 27
JULY 12
JULY 24
100
50
0
% MORTALITY
h=hours
d=days
T
C
1 h
8 h
12 h
24 h
3 d
5 d
7 d
12 d
13 d
TIME POST TREATMENT

Figure 12. The per cent mortality of horse flies (spp.) used in the bioassay for spray periods 3 and 4 at the Seven Sisters Grassland Project, Manitoba, 1984.


HORSEFLIES
JULY 12
100
50
0
T
C
h=hours
d=days
% MORTALITY
JULY 24
100
50
0
T
C
1 h
8 h
12 h
24 h
3 d
5 d
7 d
12 d
13 d
TIME POST TREATMENT

DISCUSSION

1. Biting Flies

The composition and abundance of biting flies differed from 1983 to 1984, especially with respect to the mosquito species. These differences are probably a direct result of moisture conditions in the spring and summer of 1983 and 1984. Some mosquito-breeding sites, which were a result of snow-melt and summer rains, were located directly in or bordering the paddocks and could be sampled. The vast majority of mosquito breeding probably took place in leaf-litter and sphagnum pools in the fen surrounding the study site.

The seasonal distribution of the tabanid species in this study differs slightly from 1983 to 1984. Similar data taken from Whitemouth Lake, Manitoba has been taken by Galloway (pers. communication, Appendix D) with similar results. Although the date of emergence differs by approximately 2 weeks between 1983 and 1984, the species composition is the same. There appears to be 3 'groupings' of species whose populations increase at various times during the summer. The first grouping is comprised of Hybomitra lurida, H. nuda and H. lasiophthalma. The emergence of H. lurida and H. nuda are the earliest of all tabanid species found in Manitoba (Hanec and Bracken 1964). Hybomitra lasiophthalma also appears quite early in the

season, but, lingers on throughout the season. The second grouping of tabanids is H. affinis, H. zonalis and H. epistates which appear in late June and early July. The last grouping of tabanids is H. pechumani, H. trepida and H. frontalis. These species peak in mid-July with H. frontalis being the last to disappear. This generalization involves only those species which were abundant at the study site.

The emergence of tabanids for other geographic areas of the northern Nearctic region is similar although the distribution and seasonal abundance of certain species may by affected by annual variation in climate and differences in soil and faunal types (Hanec and Bracken 1964). Teskey (1969) in his comprehensive study of the immature stages of Tabanidae summarizes the distribution of Hybomitra as mainly northern, where the habitat substrata are primarily organic. Some species of Hybomitra and Tabanus seem to have preferences for restricted habitats (Teskey 1969), based upon moisture level, acidity, stream currents, etc. While still others seem adapted to a wide variety of suitable conditions.

The more common species of Hybomitra in collections from nothern Ontario (Kenora and Patricia districts), were active at similar times as they were at the Seven Sisters Grassland Project (Pechuman et al., 1960). Baribeau and Maire (1983) compared the seasonal distribution and abundance of tabanids in temperate versus subarctic Quebec. In the temperate

location, H. lasiophthalma and H. pechumani were among the most abundant of the 43 species collected with Chrysops aestuans being the most abundant. Tabanids were active from mid-May to mid-August with the peak from mid-June to mid-July, similar to this study. In the subarctic region, however, the most abundant species was H. arpadi. Tabanids were active from the 2nd week of July to the 1st week in August. In New York State, the seasonal distribution of these species is spread over a longer period of time (Pechuman and Burton 1969), presumably because of the milder climatic conditions.

All of the more common species of Hybomitra were more numerous in 1984 than in 1983 according to the catches of the MHFTs. Although the design of the MHFT differed from 1983 to a more efficient trap in 1984, there were notably more flies observed around the animals in 1984. It is unknown why populations fluctuate from one year to the next; it is probably a result of a combination of factors such as larval habitat conditions, available sources for blood meals, and sufficient habitat for oviposition. Since 1980, when monitoring of biting-fly populations began at this site, the populations of tabanids have been fluctuating yearly, but populations have been reduced relative to past years at the project (Galloway pers. communication). This decline may be the result of one or a combination of several factors: 1) extensive peat farming within 2 km of the

study site may be destroying the habitat of tabanids; 2) the water table of the area may be decreasing, reducing suitable habitat for the larvae; 3) continual attempts of chemical control of the flies for 7 consecutive summers at the study site may be reducing the adult populations each year.

The MHFT supplemented with 4.45 kg of dry ice doubled the number of tabanids trapped. Increased numbers of tabanids trapped with the supplementation of dry ice has also been observed by Burger (pers. communication, 1984). Malaise traps supplemented with carbon dioxide attracted up to 4 times more tabanids than unbaited traps (Roberts 1972; Blume et al., 1972). There was no difference in attractiveness between traps baited with CO_2 and traps baited with a steer (Roberts 1972). Since vision and the presence of CO_2 are two powerful attractants for tabanids, then it is not surprising the MHFT supplemented with dry ice attracted more flies. One interesting point is that during the two years that this study was done, the author was bitten only once. The combination of CO_2, visual contrast and movement of the larger heifers was probably a stronger attractant than a human observer.

2. Weight Gains

Similar studies to this one, in which the impact of biting flies was evaluated by weight gains of cattle, have yielded results with greater treatment differences (Khan and Kozub 1985; Roberts and Pund 1974; Steelman et al., 1972). However, most of these studies involve protecting cattle by means of chemical application, and thus protection is only as good as the insecticide. Although cypermethrin is an effective insecticide, as indicated by the bioassay, there appears to be no repellency, and thus cypermetherin is effective in killing, but only after the flies have attempted to blood-feed. Shemanchuk (1981) found that permethrin repelled black flies longer than cypermethrin, and as an ethanolic solution, cypermethrin did not repel the flies before they could bite and take a partial blood-meal. Therefore, it seems treated animals are equally susceptible to being fed upon as are the control animals. Perhaps this might explain why there was no significant difference (P=.05) in the weight gains between control and treated herds. Horse flies were more susceptible to cypermethrin than mosquitoes in bioassay experiments. However, this may be a result of the amount of time horse flies and mosquitoes were directly exposed to the hair coat treated with cypermethrin. Horse flies were exposed to a lethal dosage of cypermethrin through their continuous attempts at blood feeding. Mosquitoes did not attempt to blood feed and

perhaps did not accumulate a lethal dose after 5 days post treatment. In forcing the mosquitoes down onto the hair, the rough handling of the cages may have caused the high mortality found in the controls. Further, loss of the chemical may have resulted from self-grooming, as was found with permethrin (Kinzer et al., 1983), contact with the ground, rubbing, and rain.

Biting flies did not appear to be a sufficient environmental stress to cause reduced weight gains and/or weight loss in pastured animals. Whether the populations of flies during this two year study were sufficient to cause changes in normal gains is unknown. The average daily gains for heifers on brome-alfalfa pastures is approximatley .7 to .9 kg (1.5 to 2.0 lbs) (R.J. Boila pers. communication). Since the gains experienced during 1983 and 1984 were .82 to 1.03kg respectively, this might imply that there was not sufficient environmental stress to affect weight gains. The most detrimental flies at Seven Sisters in terms of reactions by the heifers, were the tabanids, in so much as one tabanid could cause a response. During crepuscular times, the animals were agitated by the mosquitoes, especially early in 1983 when the spring Aedes were abundant. Although the animals were noticeably bothered, during the day by tabanids, and at dusk and dawn by mosquitoes, perhaps the energy expended by the animals as avoidance behaviour did not affect weight gains.

Another factor which can control weight gains, is the pasture itself. In 1983, the percent crude protein of the pastures was 10.6% (orchard grass, Timothy, alfalfa) and in 1984, the pastures were 13.5% (Timothy, alfalfa) (E.G.S. Annual Report 1982). By the NRC standards (1978) for dairy cattle, expected average daily gains for heifers 200 - 250 Kg should be 200 - 800 gm (.7-1.7 lbs). Since the paddocks in 1983 and 1984 were divided to produce pastures A and B, each pasture should be approximately equal in nutrient value. However, in both years, a pasture bias was noticed, the reasons for which are unknown.

This experiment was designed to reduce variation in weight gains by altering the herds in pastures A and B each spray period. However, this design seems to be unique when compared to other weight-gain trials dealing with the impact of flies. In a feedlot situation, steers can be confined in pens, the advantage being that control animals can be screened from biting flies, yet animals are under the same environmental and nutritional conditions as the exposed animals (Perich et al., 1986; Steelman et al., 1972, 1973). This design is impractical in a pasture situation. If an experiment is designed using different locations for treated and control experimental units (Bailie and Morgan 1980), differences in weight gains may be a result of different fly species, pasture conditions and management practices. Efficacy trials with ear tags controlling horn flies are

done on pasture, but the animals are not switched on pasture to reduce pasture bias (Quisenberry and Strobehn 1984; Harvey and Brethour 1983). Although impossible in this study, replication can be obtained by subdividing the paddock areas into suitable pastures, thus further reducing the effects of pasture bias.

CONCLUSION

There were no significant differences in weight gains due to treatments in either 1983 or 1984. This may be due to one of two factors: the fly pressure in either year was not sufficent to cause an environmental stress resulting in reduced weight gains or the chemical, cypermethrin has no repellency effect, and thus, flies attacked the treated animals with the same consistency as the control animals. Cypermethrin cannot be labelled as ineffective since results of the bioassay indicate mortality of mosquitoes and tabanids from exposure to the chemical. Populations of tabanids and summer Aedes peak during the second and third week of July in this area of Manitoba. Thus, control measures of the flies on pastured animals might be more effective if concentrated during this time. The fact remains, however, that there is no effective method for controlling tabanids on pastured cattle.

THE EFFECTS OF BITING FLIES ON INDIVIDUAL AND GROUP BEHAVIOUR OF DAIRY HEIFERS

INTRODUCTION

The daily and seasonal activity patterns of pastured cattle can be greatly influenced by increased populations, and species, of blood-sucking flies. In Manitoba, this is particularly true at a time when producers expect maximum gains of pastured animals. Harassment of the animals and changes in daily activity are of concern because irritation can cause a reduction in grazing and proper rumination, resulting in reduced weight gains and milk yeilds. Daily behavioural patterns can be considered as interactions between the animal and its surrounding environment (Hafez and Lindsay 1965). The objective in this study was to observe changes in the behaviour of pastured dairy heifers as influenced by biting flies.

Some biting flies are distributed among cattle depending upon characteristics of the host such as coloration and sex (Brown and Bennett 1980). Variation in attractiveness of each animal can result in variation of behaviour patterns of individuals when attacked by biting flies. Consequently, it is more advantageous to use an intermittent rather than a continuous recording of behaviours (Hull et al., 1960), allowing more animals to be observed in each treatment-group (Hafez and Lindsay 1965). 'Instantaneous' sampling, as described by Altmann (1974), allows for the recording of behaviours at preselected periods of time and can provide information about the frequency and time spent displaying a

particular activity. Information obtained from 'continuous' (Altmann 1974) sampling of one individual can be extremely biased since the chosen individual may not reflect the typical behaviour of the rest of the herd.

Cattle are diurnal feeders (Houpt and Wolski 1982) and behaviour is influenced by environmental stresses such as forage quality, temperature, or flies. In cattle, the major periods of grazing are at sunrise and sunset with some grazing occurring at midmorning and early afternoon. Five to seven hours per day may be spent grazing, but it is inversely porportional to the quality of the pasture, and reflects the nutritional needs of the animal (Arnold and Dudzinski 1978; Hart 1985). Grazing activity is alternated with walking, resting, and rumination (Hart 1985). At high temperatures, cattle will move to the shade, and generally the time spent grazing during the hotter periods of the day decreases (Arnold and Dudzinski 1978). There are few studies concerning the behavioural changes due to environmental stress on pastured cattle, which include the effects of biting flies (Fraser 1985). If the behaviour of grazing animals is important to the utilization of pasture (Kiley-Worthington 1977), and therefore animal health and production, then the seasonal factor of biting flies should not be overlooked.

The objective of this study was to examine the variation of host behaviours between a control herd and a similar herd

of dairy heifers treated with cypermethrin. Group responses such as aggregation associated with insect activity were also noted.

MATERIALS AND METHODS

Treatments

Forty dairy heifers in 1983 and 34 dairy heifers in 1984 were supplied by various producers in eastern Manitoba. The age and weights of the animals ranged from 9 to 17 months, 212 to 386 kg in 1983 and 8 to 16 months, 153 to 320 kg in 1984. The animals were equally divided into 2 herds, and although there were mixed breeds of heifers in both years, each breed was equally represented in either herd. Each animal in the treated herd received a 2L, full-body-spray of 0.1% (2gm a.i.) cypermethrin in 1983, and 1L of a 0.1% (1gm a.i.) cypermethrin in 1984. The treated animals were sprayed and all animals weighed biweekly. Each two-week period between sprays was referred to as a spray period, of which there were 4 in 1983 and 3 in 1984. In 1983, the animals were pastured in paddock 1, pastures 1 and 2 (Figure 1, Manuscript 1). These pastures had a transecting tree line which provided shade. In 1984, the animals were pastured in paddock 5, pastures 1 and 2 (Figure 1, Manuscript 1), where no shade was available. The treated and control herds were alternated between pastures during each spray period to reduce pasture bias.

Biting Flies

Data concerning the species and populations of biting flies and their effect on animal behaviour were obtained in conjunction with the weight gain study (Manuscript 1). Many of the materials and methods for the behaviour study were the same, so for a complete explanation refer to Manuscript 1. The methods presented here emphasize how the biting fly data were collected for the behaviour study.

Populations of biting flies were monitored daily using a NJLT for mosquitoes and MHFTs for horse flies. Mosquitoes and horse flies were collected, sorted and identified the same day they were trapped. Populations of black flies and stable flies were not quantified but casual observations were made on their abundance and behavioural effects on cattle. During field observations of behaviour, landing patterns, site selection, and abundance of certain species were also noted.

Behaviour

The behaviour of the animals was observed for both 1983 and 1984. The animals were monitored twice weekly and the frequency of annoyance variables such as ear flicks, tail switches, head tosses, and foot stomps recorded. These variables were defined as follows:

1. Ear flicking - a single forward and backward motion of the ear or ears.
2. Foot stomping - a kick or stomp of any leg due to annoyance by insects.
3. Tail switching - movement of the tail to one side or the other so that it makes contact with the side of the body.
4. Head tossing - a movement of the head to one side of the body or the other, not necessarily contacting the body.

Observations were made for 4 hours during the peak of tabanid activity between 1100 hr and 1500 hr. Each animal was observed for one hour, during which its corresponding 'sister' (animal from the same producer) was observed in the adjacent paddock. The behavioural responses were counted and recorded for 3 out of every 10 minutes. The air temperature in the paddocks was recorded hourly. General observations were also recorded during this time and throughout the week such as, animals which were in estrus,

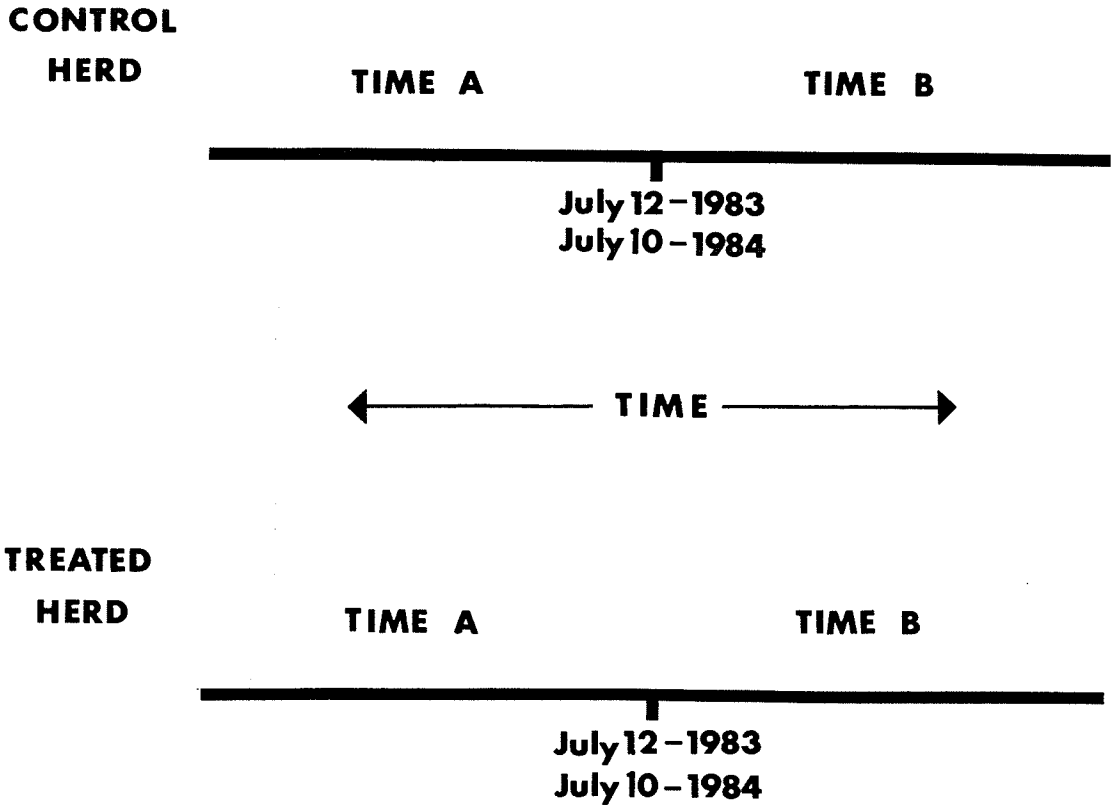
those animals which were dominant or subordinate and whether or not the flies were particularly numerous.

Twenty-four hour observations of behaviour were conducted in 1983 and 1984 for the purpose of noting any changes in daily routine. There were two twenty-four hour periods of observations in 1983; one before (9 June) and one during (14 July) the peak of the tabanid activity. Three twenty-four hour periods of observation were made in 1984; before (16 June), during (28 June), and after (2 August) the tabanid peak. The methods used for all periods of observation in 1984, as well as the observation on 14 July, 1983 were the same. Each animal was observed for one hour out of 24 and the frequency of annoyance variables recorded as described for the biweekly observations. Observations began at approximately 0700 hr and each observer was replaced after 3 hours. The 24-hour observation of behaviour on 9 June, 1983, was recorded differently from every other date. One animal was chosen randomly from each of the treated and control herds. These animals were observed for a twenty-four hour period. The location of the herds in the paddocks was recorded every 15 minutes, at which time the number of animals walking, grazing, watering and resting was also recorded. The number of head tosses and tail switches was recorded for 10 out of every 30 minutes.

Analysis

Differences of behaviour between treated and control animals were analyzed by both univariate and multivariate methods. Univariate methods consider the distribution and variation of a single variable. Multivariate analyses offer the advantage of simultaneously considering the joint distribution of the variables. Differences which are not detectable from univariate analysis may show high significance using multivariate methods. The variables in this study (head tosses, ear flicks, foot stomps, and tail switches) were transformed (natural logarithm) due to non-normality, and examined jointly since they are a representation of behaviour. Although the data were initially divided by treatments (control and treated), further division of the data was done according to time. The pasture season was divided into 2 periods; before and after 12 July (1983) and 10 July (1984). These dates were chosen as the points which separated low populations (first half of the fly season) from high populations (second half of the fly season) of tabanids. Consequently, behaviour of animals can be based on time (and therefore fly intensity) and treatment (Figure 13). The joint distribution of all the variables as they are affected by treatments and time were examined, and canonical discriminant analysis was used (SAS Institute Inc., 1985). (See Appendix D for a further explanation of canonical discriminant analysis).

Figure 13. A schematic diagram of the times and treatments which make up each of the four groups. Groups are defined as distinct units possessing unique qualities. Thus, Groups 1 - 4 are all the combinations of time and treatment; time being either the beginning (A) or the end (B) of the fly season, and treatment representing either control or treated animals. Therefore, all of the daily counts fall into one of the 4 groups.



GROUP 1 = TIME A, CONTROL
GROUP 2 = TIME A, TREATED
GROUP 3 = TIME B, CONTROL
GROUP 4 = TIME B, TREATED

RESULTS

The results section is divided into 2 parts. The first is a descriptive summary of the animal behaviour from field observations, and the second is a summary of the statistical results. Data concerning fly populations and species are presented in Manuscript 1, however some of these data are included in this manuscript to support observations of the animal behaviour.

A. Field Observations

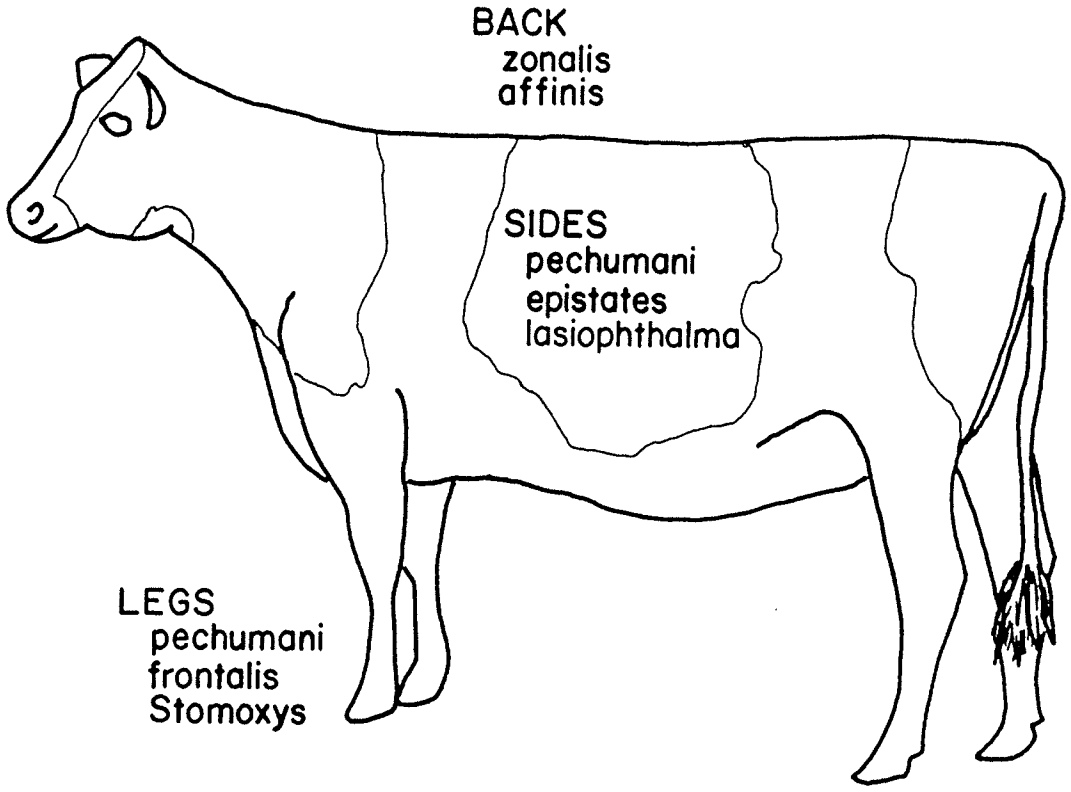
In addition to quantifying certain aspects of individual behaviour, many hours of field observations have yielded valuable information regarding the influence of biting flies on behaviour of the dairy heifers. Therefore, this section deals with site selection and orientation of the biting flies, and group and individual behavioural responses.

Landing Patterns and Host Site Selection

Specific landing patterns and site selection of Stomoxys calcitrans (L.) and those species of tabanids which were recognizable from a few meters, were observed in 1983 and 1984 (Figure 14). Horse flies appear during a relatively short time period in Manitoba, (mid June to mid August) (see Manuscript 1), and thus populations of most species peak and

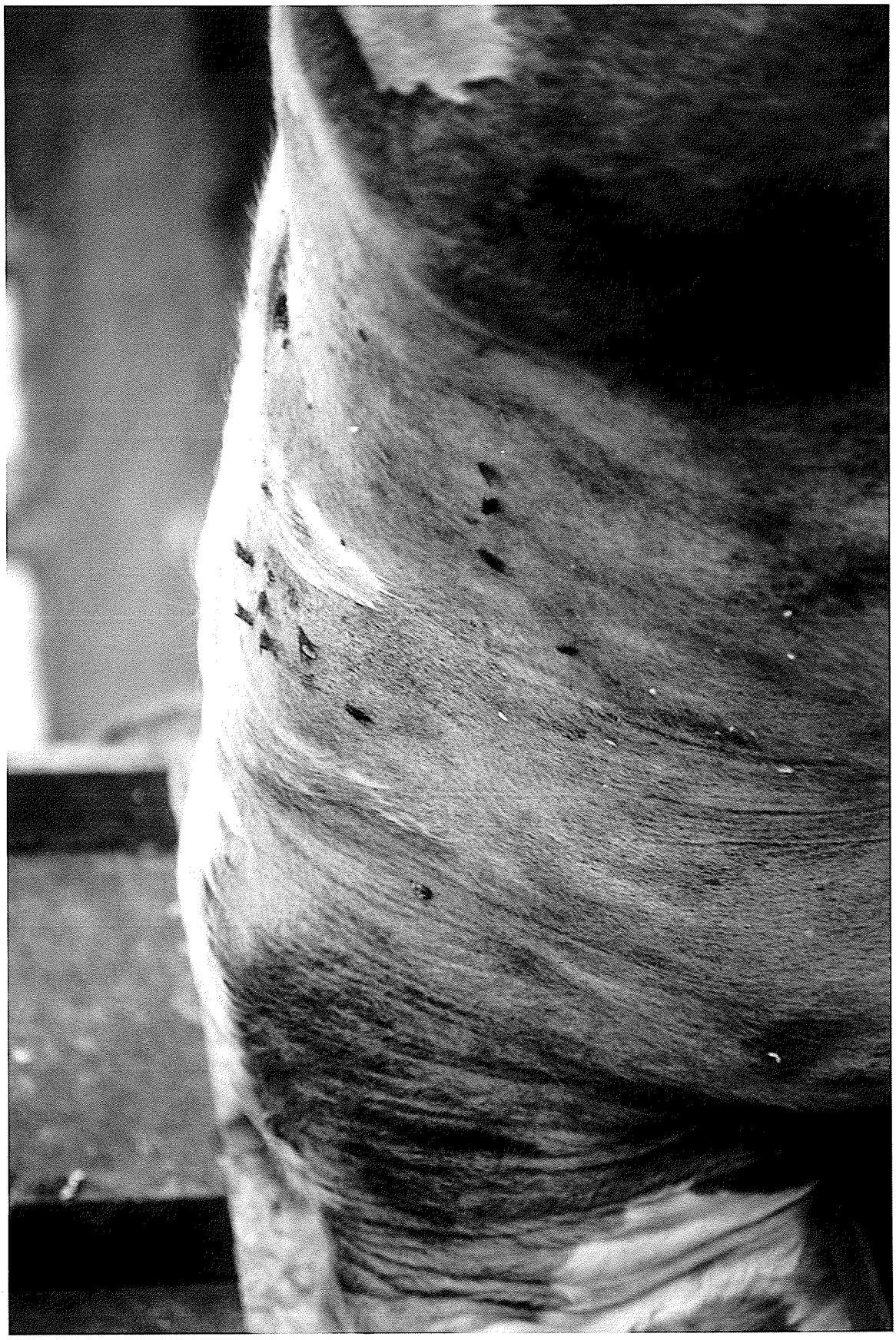
Figure 14. Preferred areas of landing and feeding by some species of horse flies and stable flies observed at the Seven Sisters Grassland Project, during 1983 and 1984.

HEAD
lasiophthalma
pechumani
epistates
nuda
lurida



overlap. However, it was observed that some of the recognizable species were attracted to and consistently landed on specific areas of the heifers. The most noticeable of the horse flies was Hybomitra zonalis (Kirby), which fed primarily along the back line. Hybomitra affinis (Kirby) also fed along the back line (Figure 15), and since H. zonalis and H. affinis occurred at approximately the same time, both species were sometimes observed feeding together. Hybomitra nuda (McD.), H. lurida (Fallen) and H. lasiophthalma (Macq.) were the first species of tabanid to appear in the season and fed primarly on the head. Although populations of H. nuda and H. lurida had virtually disappeared by the beginning of July, H. lasiophthalma lasted until August and continued to feed primarily on the head, but also along the sides of the heifers. Populations of H. epistates (O.S.) peaked approximately one week after H. nuda and H. lurida had disappeared, and, along with H. lasiophthalma, fed primarily on the head and sides. Hybomitra pechumani Teskey and Thomas occurred over most of the body except the belly region, but the primary feeding areas were the face, sides, and legs. Hybomitra frontalis (Walker) was the last species to peak (mid to late July), and oriented and fed on the legs. Similarly, Stomoxys calcitrans consistently oriented low on the body and fed primarly on the legs. Stomoxys calcitrans fed quite readily on the sides, neck, and legs of an Angus bull which was located in a pasture adjacent to the dairy heifers. On a

Figure 15. Feeding of Hybomitra zonalis and H. nuda on a feedlot steer. These horse flies fed primarily along the back-line where it was very difficult for the animal to dislodge them.



few occasions, Simulium vittatum Zetterstedt fed on, and in the ears of the heifers. Mosquitoes were not observed to exhibit specific landing patterns during crepuscular periods. However, during hot, sunny periods, mosquitoes would feed on the shady side, or underside of the animals grazing near the forested boundaries of the paddocks.

In this study, the orientation of tabanids on their hosts seemed to be greatly influenced by coat coloration. Holsteins, which had a high percentage of a black coat, were much more bothered and harassed by tabanids, although this was not measured. White Holsteins with small patches of black in their coats were not as harassed by tabanids, though the tabanids oriented and persistently landed on the black patches. The Jerseys, Brown Swiss x Jerseys, and the Red Holstein were not as harassed as the black and white Holsteins, and during severe tabanid attack, these animals were observed resting or ruminating away from the herd.

Individual Behaviour and Comfort Movements

The dairy heifers displayed specific behaviours both as individuals and as a group to dislodge or avoid biting flies, particularly horse flies (Table 5). Tail switching was the most noticeable individual response. This display was noticed during most daily activities and particularly

Table 5. A summary of the individual and group behavioural responses observed in cattle to biting flies at the Seven Sisters Grassland Project, Manitoba, 1983 and 1984.

Behavioural Response	Description
A. Individual	
1. Tail Switching	Most noticable individual response. The tail was very effective at dislodging any flies attempting to feed along the sides of the body.
2. Ear Flicking	This reaction was very frequent when the animals were grazing and at times when flies attempted to feed on the face, head and ears.
3. Head Tossing	This reaction was observed more frequently when the animals were lying down and it was used to brush flies from the sides of the body.
4. Head Shake	Very similar to head tossing, however this reaction was observed when flies were feeding on the face and head but could not be dislodged by ear flicking.
5. Licking	Along with head tossing, the animals would sometimes use their tongue to lick tabanids and mosquitoes from the body sides.
6. Foot Stomping	Stomping of the feet was seemingly a direct response to biting flies but was not entirely successful in dislodging them, especially Stomoxys calcitrans L.
7. Kicking	An accurate, directed response to biting flies feeding on the belly region.
8. Kicking or Tossing Debris	This behaviour was observed more with the bull and finishing steers. Mud, dust or grain was kicked, or scooped up by the front feet, or head, and tossed on the sides and back in an attempt to remove biting flies.
B. Group or Herd	
1. Bunching	This reaction was observed during intense mosquito and tabanid activities. Grazing became erratic and movement within the pastures increased.

Table 5. continued....

Behavioural Response	Description
B. Group or Herd	
2. Grazing Lines	During the peaks of biting fly activities, the animals would align side-by-side and begin grazing in a linear configuration.
3. 'Stampeding'	This activity was rarely observed and was caused more frequently by mosquitoes in 1983.

when populations of biting flies were high. The tail could reach approximately midway along the body and thus dislodge any flies attempting to feed in these areas. Ear flicking was also a high frequency and rapid response to biting flies, especially during grazing. Flies on the face and upper neck usually could be brushed off by ear flicking. Horse flies in flight around the head would usually annoy the animals enough to cause ear flicking, head tossing or head shaking. Tabanids which landed around the mouth were quickly licked and sometimes eaten. Stomping of the feet or directing a kick to the belly region were responses to biting flies. The kicks were usually very accurate and successful in dislodging horse flies and stable flies. These flies continued feeding on the legs even though the animal was stomping its feet. Only after the legs were brushed with the side of the head or licked, did the flies leave.

Group or Herd Responses

Since these dairy heifers originated from different producers and therefore different management practices, initial herding was quite disjointed. Initially in 1983, animals would only herd during bouts of grazing; resting and rumination was done with their 'sister' along the fence-line. After approximately one week, animals in the respective treated and control herds became more cohesive in

their daily activities and a social hierarchy was visible. For example, grazing, watering and resting were done as a herd rather than individually. Dominance amongst the animals was established, usually by weight and age (Reinhardt and Reinhardt 1975), and was shown by butting of subordinate animals during grazing and watering.

Once the animals were established as a herd, grazing, resting, ruminating and watering were done together, however, there were a few exceptions. For example, animals in estrus displayed conspicuously different behaviour from the rest of the herd such as running away from the rest of the herd, bellowing, and mounting other animals. In 1984, the Jersey heifers did not seem to fit into the social hierarchy of the herd. Although they were usually involved in the same activities as the Holsteins, the Jerseys and Brown Swiss - Jersey crosses were sometimes separated from the rest of the herd.

Grazing began at approximately 0545 hr to 0630 hr in June and July, 1983 and 1984. The animals were quite dispersed throughout the pasture at this time and grazing was very intensive. Mosquitoes were not as numerous in the morning as they were in the evenings, but this was dependent upon the ambient temperature. The first horse flies began landing on the animals at approximately 0830 hr, but this was also dependent upon general weather conditions. For example, very few horse flies were observed on cool or rainy

mornings. At this time, the heifers began to herd together and remained so until nightfall. The animals grazed approximately 4 to 7 times during the day, and bouts lasted from approximately 30 minutes to 3 hours. Host seeking and feeding by the horse flies peaked from approximately 1100 hr to 1500 hr and this activity increased at higher temperatures. Cloud cover, wind and low light intensity resulted in decreased flight and feeding behaviour of horse flies. Grazing in the evening usually ended at nightfall (2145 hr to 2230 hr), however on a few occasions, grazing did not terminate until 2330 hr to 2400 hr.

Influence of flies on group behaviour

Changes in group behaviour were observed during the peak of mosquito and horse fly attack. Grazing was the most noticeable daily activity affected by biting flies, especially tabanids. During intensive feeding by flies, individuals in the herd would align parallel to each other in a tight formation, or grazing line (Figure 16). As grazing proceeded across the pastures, subordinate animals would be left on the ends or left behind. Once an animal was stressed by biting flies and became intolerant, it would push and shove for an internal position in the line. Thus, the line was continually changing shape and direction. This type of behaviour was only noticed in 1984. In times of

Figure 16. The formation of grazing lines was quite common during peak tabanid activity. Each animal would align in the same direction and stay close to the animal next to it. The line would move and change direction as grazing proceeded around the pasture. (courtesy of T. D. Galloway)

tabanid attack during 1983, the animals took shelter, rested and ruminated in the trees.

Another group behaviour which was noticed during severe tabanid attack, especially by Hybomitra pechumani, was bunching (Figure 17). If the grazing lines broke down due to the intensity of biting flies, or if the flies were too severe for the animals to lie down and rest, the animals began to bunch. Subordinate animals were usually pushed to the exterior of the bunch with the dominant animals in the middle. Animals on the exterior would push their way to the middle of the bunch and thus the group appeared to be in constant agitation. Movement as a group increased around the pasture with some attempt at grazing. At times the animals would lie down (Figure 18), but within minutes the flies became intolerable and the animals would resume a position in the grouping. This type of behaviour was noticed throughout the season but was more prevalent during tabanid attacks.

The last group behaviour which seemed to be a direct result of biting flies was 'stampeding'. This was only noticed in 1983 during severe feeding by mosquitoes. Mosquitoes were easily disturbed by cattle walking or grazing, and animals grazing in the west end of paddock 1 during crepuscular periods were extremely agitated by the spring Aedes spp. When the mosquitoes became intolerable, the animals would begin to run back to the tree line for

Figure 17. During intensive feeding by tabanids (H. pechumani), grazing would stop, and the animals would form tight bunches for maximum protection against the flies.

Figure 18. Animals which tried to lie down during intense attacks by tabanids were severely fed upon. Animals quickly became intolerant of this, would stand and join the rest of the herd.

shelter. Once an animal began to run, the rest of the herd followed. This alternating behaviour of grazing, and running for shelter, continued until the animals bedded down for the night.

B. Statistical Results

Treatment differences for each behavioural variable in 1983 and 1984 were tested using the Student's T-test (Table 6). Observations in which an animal was lying down were removed from the data set. In 1983, tail switches and foot stomps were significantly higher in the control group than the treated (P=.05). In 1984, the number of head tosses in the treated group was significantly higher than the controls (P=.05).

There was a significant difference among the groups 1, 2 3, and 4 (Figure 13) in 1983 ($F(3,302) = 12.62$, P=.05) and 1984 ($F(3,439) = 37.16$, P=.05) (MANOVA). Classification of the variables into each of the 4 groups is summarized in Table 7. In 1983, the highest percentages of observations into the groups were those which were already assigned to that group; 37.7% were classified into Group 1, 46.5% were classified into Group 2, 50.0% were classified into Group 3, and 35.3% of the observations in Group 4 were classified into Group 4. In 1984, Groups 1 and 4 had the highest percentage of observations which were assigned to their own

Table 6. Student's T-test and significance of treatment differences for each behavioural variable (natural logarithm value) in 1983 and 1984, for the daily count data.

1983

Variable	Trt[a]	N	Mean[b]	Std Err of Mean	T Statistic	df
Lear	0	157	2.4457	0.1129	0.9877[ns]	297
	1	150	2.3009	0.0934		
Lhead	0	157	0.8988	0.0764	0.8248[ns]	305
	1	150	0.8023	0.0888		
Lfoot	0	157	1.5386	0.0844	3.3663[*]	305
	1	150	1.1245	0.0896		
Ltail	0	157	3.8850	0.0896	3.3960[*]	299
	1	150	3.4847	0.0765		

1984

Variable	Trt[a]	N	Mean[b]	Std Err of Mean	T Statistic	df
Lear	0	188	3.2680	0.1058	0.9611[ns]	358
	1	172	3.1293	0.0969		
Lhead	0	188	0.9621	0.0885	-2.0179[*]	358
	1	172	1.2083	0.0830		
Lfoot	0	188	0.9551	0.0909	-1.4704[ns]	358
	1	172	1.1506	0.0972		
Ltail	0	188	3.5482	0.1088	-1.4519[ns]	358
	1	172	3.7729	0.1095		

[ns] denotes non-significance ($P > .05$)

[*] denotes significant differences at $P \leq .05$

[a] 0=unsprayed, control; 1=animals received 1.0 gm a.i. cypermethrin spray at biweekly intervals

[b] number of responses / 3 minutes

Table 7. The classification of each observation in discriminant analysis from its own group(information already known), to another group (to which it is most similar). The values represent the actual number of observations in each classification (top) and the percentage of the total (bottom).

1984

		Number of Observations and Percentages Classified into Group:				Total
		1	2	3	4	
From Group:	1	44 55.70	4 5.06	11 13.92	20 25.32	79 100.00
	2	23 32.39	12 16.90	13 18.31	23 32.39	71 100.00
	3	24 16.33	4 2.72	54 36.73	65 44.22	147 100.00
	4	14 9.59	2 1.37	30 20.55	100 68.49	146 100.00
Total Percent		105 23.70	22 4.97	108 24.38	208 46.95	443 100.00

1983

		Number of Observations and Percentages Classified into Group:				Total
		1	2	3	4	
From Group:	1	43 37.72	29 25.44	24 21.05	18 15.79	114 100.00
	2	19 16.67	53 46.49	28 24.56	14 12.28	114 100.00
	3	12 10.17	33 27.97	59 50.00	14 11.86	118 100.00
	4	24 20.17	35 29.41	18 15.13	42 35.29	119 100.00
Total Percent		98 21.08	150 32.26	29 27.74	88 18.92	465 100.00

groups (55.7% in Group 1, 68.5% in Group 4). The percent classifications of Groups 2 and 3 (16.9% and 36.7%, respectively) were not the highest in their own groups.

The group means, from Table 8, were plotted in canonical space, using each canonical variable as the axis (Figures 19,20). Canonical variable 3 is not represented on the graph as the amount of variation due to CAN 3 was not significant (P=.05). The Mahalanobis distance is described as the distance between the group means (G1 - G4), and the probability of a group distance being greater than the Mahalanobis distance is given in Table 9. From these probabilities for 1983, there is no significant difference (P=.05) between Groups 1 and 4. Thus, based on the behavioural variables, Groups 1 and 4 are statistically similar. Groups 2 and 3 are statistically different from all other groups. Thus, control animals in time A (Group 1), are the same as treated animals in time B (Group 4). Similarly, treated animals in time A and control animals in time B are statistically different from all other groups. The vectors on Figure 19 are plotted according to the canonical coefficients of each original variable (Lhead, Lear, Lfoot, Ltail) (Table 10) and they represent the directional 'pull' each variable has on the groups. The vector Lear is responsible to a great extent, for the horizontal separation of the groups. Thus, the significant spatial differences of Group 2, and Group 3 from Groups 1

Figure 19. The group means, G1, G2, G3, and G4, graphed in canonical space for the 1983 daily-count data. The original variables are represented by the respective vectors and are graphed according to the canonical coefficients.

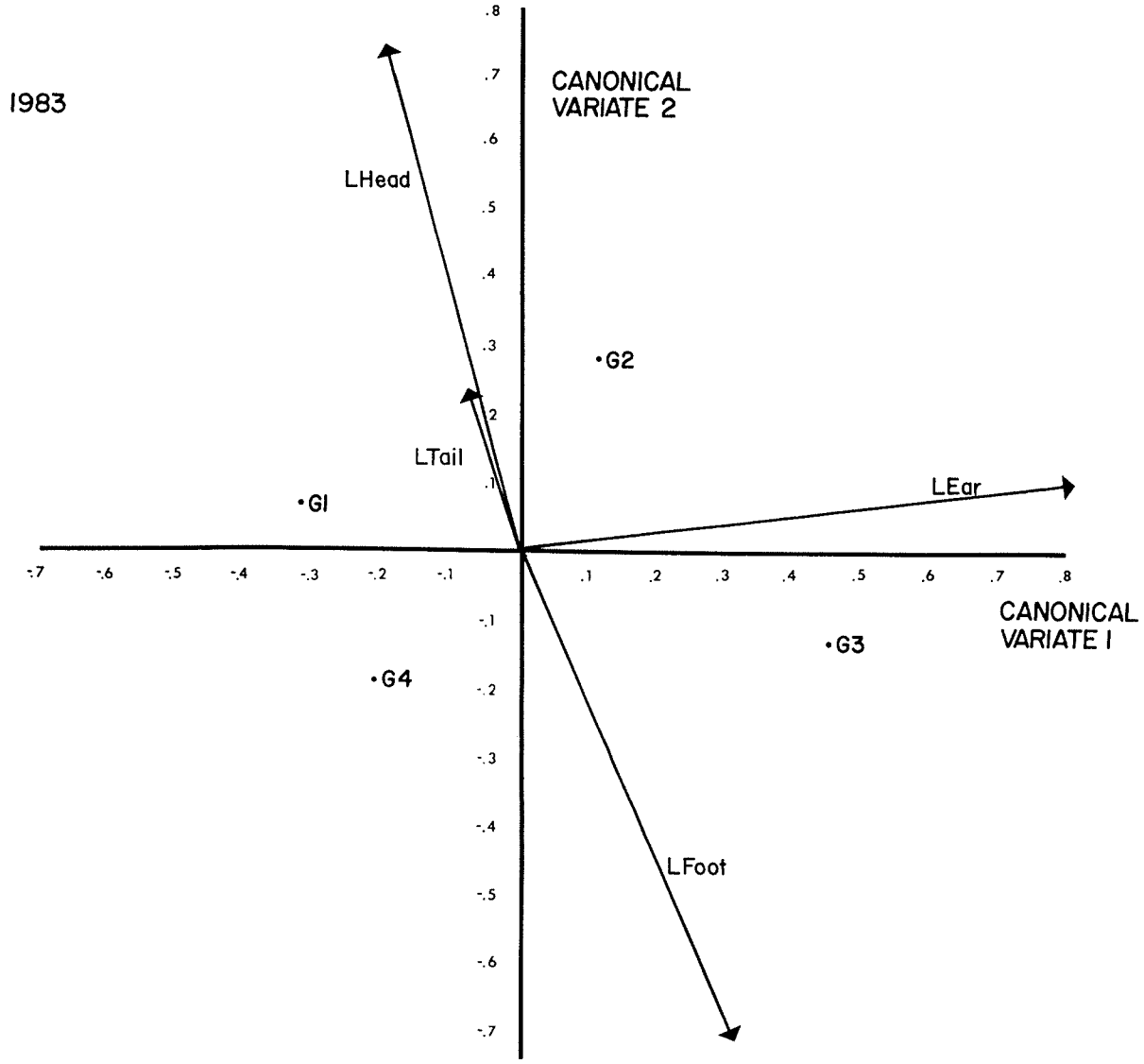

1983
CANONICAL VARIATE 2
LHead
LTail
LEar
LFoot
•G1
•G2
•G3
•G4
CANONICAL VARIATE 1
.8
.7
.6
.5
.4
.3
.2
.1
-.1
-.2
-.3
-.4
-.5
-.6
-.7
-.7
-.6
-.5
-.4
-.3
-.2
-.1
.1
.2
.3
.4
.5
.6
.7
.8

Figure 20. The group means (G1 - G4) graphed in canonical space for the 1984 daily-count data. The original variables are represented by the respective vectors and are graphed according to the canonical coefficients.

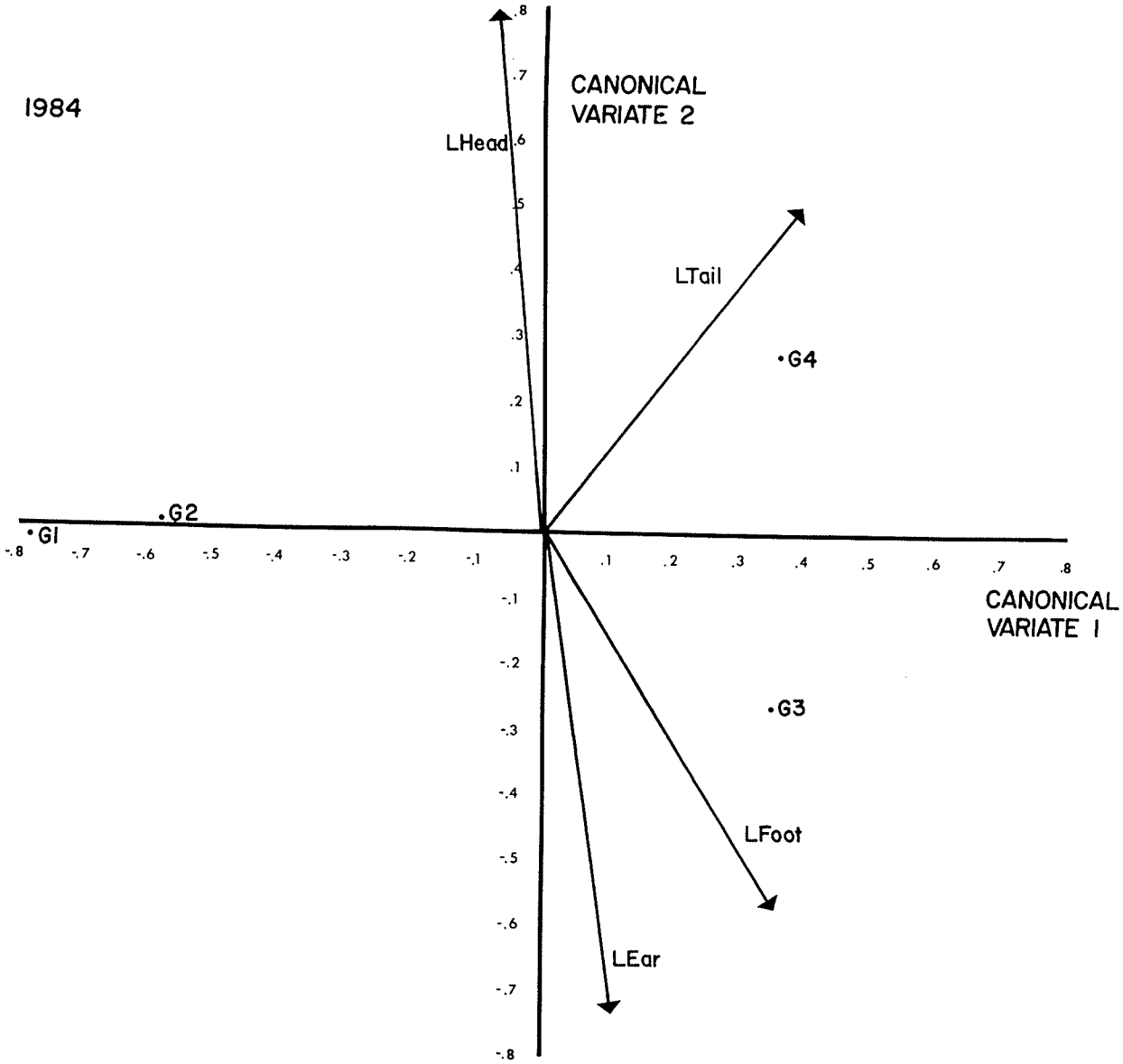

1984
CANONICAL VARIATE 2
CANONICAL VARIATE 1
LHead
LTail
LFoot
LEar
•G1
•G2
•G3
•G4
.8
.7
.6
.5
.4
.3
.2
.1
-.1
-.2
-.3
-.4
-.5
-.6
-.7
-.8

Table 8. The group means on each canonical variable for the daily count data during 1983 and 1984 at the Seven Sisters Grassland Project, Manitoba.

1983

	CAN 1	CAN 2	CAN 3
Group 1	-0.3206	-0.0650	0.1109
2	0.1135	-0.2783	-0.0672
3	0.4552	0.1331	0.0438
4	-0.2529	0.1969	-0.0853

1984

	CAN 1	CAN 2	CAN 3
Group 1	-0.7961	0.0062	-0.1370
2	-0.5840	-0.0157	0.1867
3	0.3501	0.2695	-0.0048
4	0.3623	-0.2671	-0.0119

Table 9. The probability of distance of the group means being greater than the Mahalanobis distance (between group means). If the probability is 5% or less that a group distance is greater than the Mahalanobis distance then the distance is highly significant (a=.05).

1983

	Probability Mahalanobis Distance			
Group	1	2	3	4
1	.	0.0051	0.0000	0.1520
2	0.0051	.	0.0015	0.0003
3	0.0000	0.0015	.	0.0000
4	0.1520	0.0003	0.0000	.

1984

	Probability Mahalabobis Distance			
Group	1	2	3	4
1	.	0.2876	0.0000	0.0000
2	0.2876	.	0.0000	0.0000
3	0.0000	0.0000	.	0.0004
4	0.0000	0.0000	0.0004	.

Table 10. The canonical coefficients (weights) for each response variable of the daily count data for 1983 and 1984, at the Seven Sisters Grassland Project, Manitoba.

1983	CAN 1	CAN 2	CAN 3
Lhead	-0.2071	-0.7235	-0.0193
Lear	0.7989	-0.1069	-0.3698
Ltail	-0.7567	-0.2104	0.8744
Lfoot	0.3161	0.7034	-0.2999

1984	CAN 1	CAN 2	CAN 3
Lhead	-0.7521	-0.9531	0.2957
Lear	0.1186	0.7197	-0.3725
Ltail	0.3821	-0.4821	-0.4248
Lfoot	0.3541	0.5639	0.8340

and 4 are mostly due to the variable Lear. Groups 2 and 3 have large numbers of Lear counts as compared to Groups 1 and 4. The vector Lfoot, to a large extent, is responsible for the vertical separation between Groups 2 and 3 since there is no significant difference between Groups 1 and 4. Since the vector is directed downwards and roughly towards Group 3, Group 3 has a larger number of these counts than Group 2. Vectors Lhead and Ltail also contribute to this vertical separation, but in the opposite direction, and Ltail is of a lesser magnitude.

The distance between Groups 1 and 2 was not significantly different in 1984 (Figure 20), but the distances from Groups 3 and 4 to all other Groups is significantly different. Biologically, Groups 1 and 2 represent the same time of the season (time A), but different treatments. Similarly, Groups 3 and 4 represent the same time (time B) and different treatments. Therefore, based upon the behaviour variables, animals at the beginning of the season react the same regardless of treatment. Animals at the peak of tabanid activity not only react differently from animals at the beginning of fly season, but they also react differently depending upon treatment.

The vectors in Figure 20 are indications of the 'pull' on the groups. Ltail and Lfoot are partially responsible for the horizontal spatial separation between Groups 1 and 2, and Groups 3 and 4. Similarly, the spatial separation of

Group 3 from Group 4 is primarily due to the vertical pull of Lhead and Lear, where Group 4 would have a higher number of Lhead counts and Group 3 would have a higher number of Lear counts.

Because of the consistency with which the data were collected in 1984, the daily counts were analyzed using the Student's T-tests. This test was done for each date, comparing treatment differences for each variable (Table 11). Times in which the animals were lying down were removed, and thus the 'N' is not equal for each date. On 16 June, or the pre-fly season, Lear and Ltail were significantly different by treatment. Date 2 (28 June) was supposed to represent the peak of fly activity for the season, however, this date was approximately 2 weeks ahead of the actual peak. Significant treatment differences for Lfoot were observed at this date. At the end of the fly season, (3 August), significant treatment differences for Lhead were observed.

The purpose of the 24 hour counts was to determine whether the flies affected the daily routine of the animals. Therefore, the activity for each 3-minute count was calculated as a percentage of each 24-hour observation for the treated and control herds (Table 12). The daily activities were divided into grazing, lying or ruminating, and standing or walking. Grazing and lying/ruminating, were consistently higher in the treated animals. The only

Table 11. Student's T-test and significance of treatment differences for each behavioural variable (natural logarithm value), for the 24-hour observations of behaviour in 1984.

Date 1 (16 June, 1984)

Variable	Trt[a]	N	Mean[b]	Std Err of Mean	T Statistic	df
Lear	0	85	0.9619	0.0613	2.3301*	157
	1	74	0.7537	0.0648		
Lhead	0	85	0.4385	0.0365	1.6719ns	157
	1	74	0.3479	0.0402		
Lfoot	0	85	0.3441	0.0411	1.8029ns	157
	1	74	0.2426	0.0375		
Ltail	0	85	1.1975	0.0615	3.2352*	157
	1	74	0.8999	0.0687		

Date 2 (28 June, 1984)

Variable	Trt[a]	N	Mean[b]	Std Err of Mean	T Statistic	df
Lear	0	121	0.8558	0.0628	-0.3681ns	234
	1	115	0.8895	0.0661		
Lhead	0	121	0.1764	0.0244	-1.1403ns	234
	1	115	0.2167	0.0255		
Lfoot	0	121	0.2514	0.0311	2.9569*	219
	1	115	0.1367	0.0231		
Ltail	0	121	0.9563	0.0647	0.5258ns	234
	1	115	0.9085	0.0636		

continued.....

Table 11 (cont.)

Date 3 (3 August, 1984)

Variable	Trt[a]	N	Mean[b]	Std Err of Mean	T Statistic	df
Lear	0	140	0.8036	0.0644	-1.4995[ns]	276
	1	138	0.9353	0.0595		
Lhead	0	140	0.1730	0.0236	-2.1841[*]	276
	1	138	0.2515	0.0271		
Lfoot	0	140	0.3107	0.0317	-0.3383[ns]	276
	1	138	0.3267	0.0352		
Ltail	0	140	0.9786	0.0686	-0.2253[ns]	276
	1	138	1.0011	0.0722		

[ns] denotes non-significance ($P > .05$)

[*] denotes significant differences at $P \leq .05$

[a] 0=unsprayed, control; 1=animals received 1.0 gm a.i. cypermethrin spray at biweekly intervals

[b] number of responses / 3 minutes

Table 12. Behavioural activities as a percentage of daily totals for 3 dates in which 24-hour observations were done, comparing the treated and control herds. Not all of the daily totals add up to 100% since other activities (e.g. watering) were not included in Grazing, Lying/ Ruminating nor Standing/Walking.

		% of Daily Total	
		Treated	Control
GRAZING	Date 1[a]	46	39
	Date 2[b]	51	48
	Date 3[c]	31	26
LYING/ RUMINATING	Date 1	46	28
	Date 2	41	42
	Date 3	48	44
STANDING/ WALKING	Date 1	8	29
	Date 2	8	10
	Date 3	16	22

[a] 16 June; [b] 28 June; [c] 3 August, 1984

exception was in the activity, lying/ruminating for date 2, when the percentages were virtually the same. Standing/walking was consistently higher in the control herd for all 3 dates. These daily percentages for activities of resting (lying/ruminating) and feeding (grazing) were higher in the treated herd, and walking/standing (including bunching) was higher in the control herd.

DISCUSSION

The importance of the results in this study do not lie in the changes of individual behavioural responses, although this is certainly valid and important information, but rather in changes of the herd response. The greatest visible impact of biting flies on pastured dairy heifers was their effect on group activities. This statement is supported by the observational studies of behaviour. In similar studies where individual behavioural responses were recorded (Okumura 1977; Harvey and Launchbaugh 1982), significant treatment differences were found. These treatment differences are, in part, supported by the comparison of individual responses between treatments, however, not all individual behavioural responses showed significant differences. This may be due to the type of response measured (i.e. head tosses, foot stomps, ear flicks, tail switches). These responses may not reflect

behavioural changes due to biting flies, when measured separately. In fact, populations of biting flies may not have been sufficient to cause significant treatment differences in behavioural changes. Finally, the chemical, cypermethrin, as indicated by the bioassay (Manuscript 1), was effective in killing flies, but was also a poor repellent, and thus, treatment differences could not easily be distinguished. Shemanchuck (1981) found that cypermethrin did not repel black flies before they could take a partial blood meal. Therefore, it would appear that cypermethrin does not sufficiently protect pastured animals.

The purpose of the 24-hour observations of behaviour, was to observe changes in daily routine of the animals. Thus, one 24-hour observation was planned before (16 June), during (28 June), and after (3 August) the tabanid peak. Although the flies were abundant during 28 June, the actual peak occurred approximately 2 weeks later. If this 24-hour observation had been done during the actual peak, treatment differences might have occurred. Similarly, the percentage of each behavioural activity for 28 June may have been different between the treatment and the control herds (Table 12).

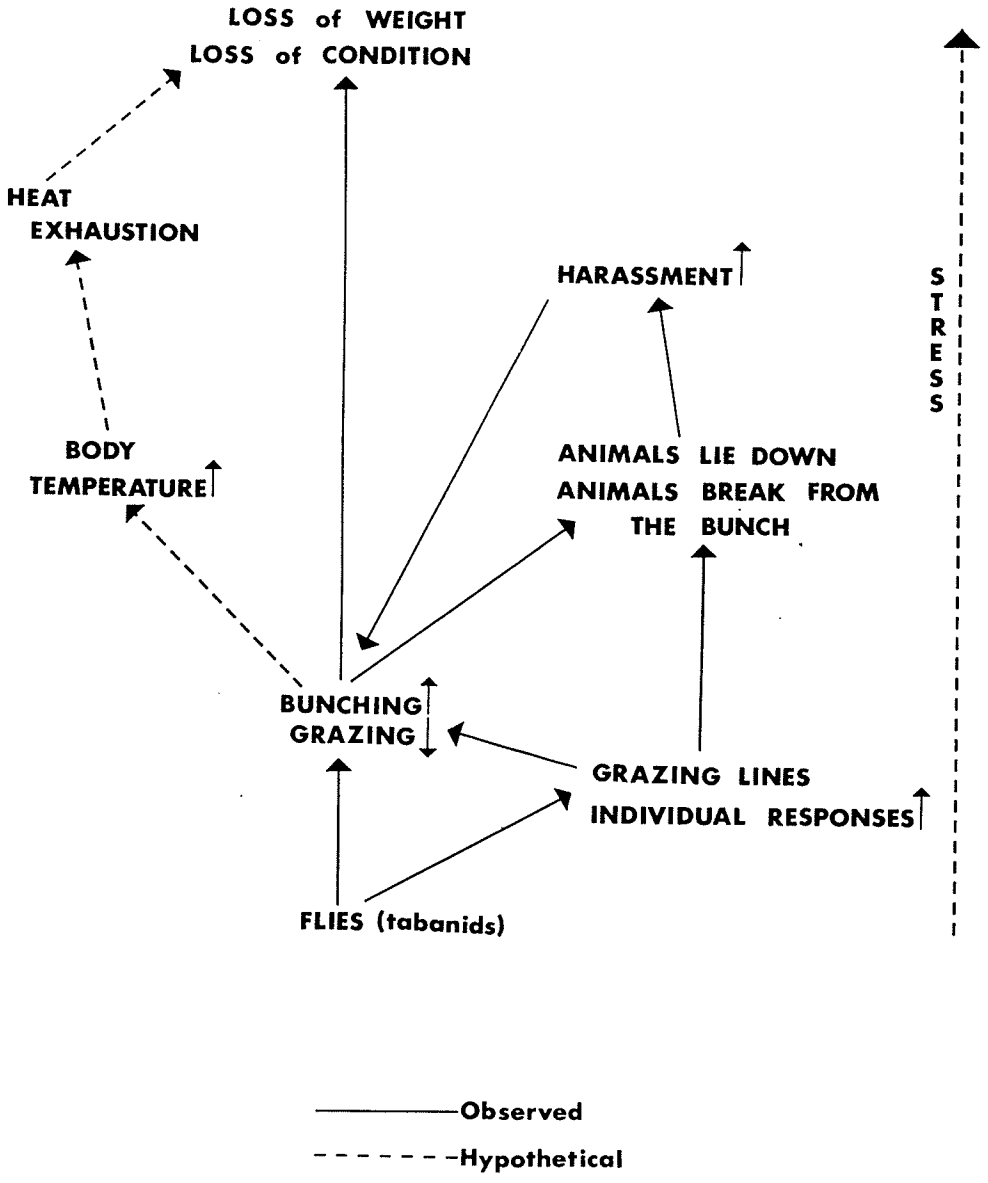
Observational Studies

It is clear from the observations in this study that horse flies and mosquitoes (especially horse flies) affect

the group behaviour of pastured dairy heifers. Although weight gains were not significantly different for the animals under the conditions of 1983 and 1984 (Manuscript 1), Figure 21 is a summary of the chain of events observed in this study which may lead to reduced weight gains. Also included are hypothetical events which may result from the types of behaviours found in this study, and may result in weight loss. Bunching may cause an increase in body temperature for animals in a crowded, tight group. This situation is compounded by horse flies being particularly active on hot, sunny days. If animals are not provided with shade, body temperature may increase to such an extent as to cause heat exhaustion resulting in loss of condition for the animals (Gwazdauskas et al., 1980). It is difficult to state whether each step in Figure 21 is an increase in stress. This can only be determined by studying the physiological processes of the animals.

Whether a reduction in grazing during the day causes a reduction in weight gains, is difficult to determine since animals will usually compensate any loss of daily grazing by grazing after dark (Arnold and Dudzinski 1978). An increase of standing and walking (as indicated by the daily counts), which includes bunching behaviour, may affect the rate of heat loss from an animal's body to the environment. Feddes and DeShazer (1986) suggest that the performance of beef cattle decreases by increased thermal stress caused by

Figure 21. The chain of behavioural events which result during severe horse fly attack. The solid lines are of events observed, and broken lines are a hypothetical chain of results.


LOSS of WEIGHT
LOSS of CONDITION
HEAT
EXHAUSTION
HARASSMENT
STRESS
BODY
TEMPERATURE
ANIMALS LIE DOWN
ANIMALS BREAK FROM
THE BUNCH
BUNCHING
GRAZING
GRAZING LINES
INDIVIDUAL RESPONSES
FLIES (tabanids)
Observed
Hypothetical

biting flies (stable flies). Production losses have traditionally been thought to be a result of blood loss and pain (Steelman 1976). Perhaps the direct effects of feeding (blood loss and pain) are secondary to the thermal stress of bunching and huddling as suggested by Feddes and DeShazer (1986). Bunching and aggregation behaviour in response to face flies have also been observed by Schmidtmann (1985), however, aggregation had little effect on thermoregulation. This may have been due to the small herd size where dissipation of body heat is probably not decreased significantly. A similar experiment could be developed in future studies involving biting flies, especially horse flies, in certain fly-infested areas of Manitoba.

Different species of biting flies and their preferred feeding sites had a direct effect on heifer behaviour. Whether the pain of biting of one species is more than another is unknown. However, if size of the fly is any indication of the pain of its bite, then the reaction towards blood feeding should be different. Hybomitra nuda is not only a large species of tabanid, but is also one of the first in the season to emerge. Since most of these animals had never been on pasture before, exposure to H. nuda and H. lurida would have been their first to biting flies. This may explain why some animals reacted to only one horse fly early in the season. Hybomitra zonalis and H. nuda feed primarly along the back-line, a place where it is

very difficult for the animals to dislodge these flies. Therefore, just a few of these flies feeding along the back-line will cause a host response. Hybomitra pechumani although a small species, appears abruptly in large numbers and feeds over most of the body. Animals react by huddling and bunching in tight groups. Although the same can be said about the size and number of mosquitoes feeding during crepuscular periods, the reaction of the animals is not the same. Mosquito activity is greatest at sunset, however, the heifers do not react by huddling and bunching, but continue grazing and increase individual responses such as ear flicking and tail switching. This may be due to the small size of mosquitoes and relatively painless bite. Stable flies, which resemble a house fly in size, orient low on the body, where they are relatively easy to brush off by the animals, and do not cause bunching or huddling. Although their bite is painful to humans, perhaps the leg area of cattle is less sensitive than other feeding sites.

Horse flies are relativly large, and their persistent attempts at feeding causes abrupt responses. Behavioural responses to the buzzing of insect flight has been observed in caribou (Rangifer tarundus L.) (Breev 1950; Espmark 1967) and black-tailed deer (Odocoileus hemionus columbianus L.) (Anderson 1975). Anderson (1975) speculated the defense response to be a 'learned' behaviour from previous encounters with bot flies. Whether this is true of

cattle responding to tabanids in flight is unknown, but a defense response has been observed when several horse flies are in flight around the ears.

Statistical Interpretation

The decision of assigning variables CAN1 and CAN2 a biological meaning is one which reflects the familiarity the researcher must have with the data. Canonical variables do not have to be directly measured or observed (Gittens 1979). In this study, the variable with the greatest variation was time; i.e., from the beginning of the season, to a chosen date, and this represented a time when the fly pressure was minimal. Indeed, the date was chosen to separate the fly pressure (tabanid) as being either intense or minimal. Data from the MHFT, numbers and species of flies (Manuscript 1, Figure 8) also correspond with the observed changes in animal behaviour. Further support of time representing CAN1, is that the cypermethrin spray did not appear to offer significant protection from flies. Therefore, treatment probably was not the variable responsible for maximum separation of the groups. Vertical separation of the groups, or CAN2, has been defined as treatment differences between the groups. Although already stated in Manuscript 1, treatment differences were not reflected in weight gains, perhaps during intense fly attacks (time B), treatment

differences are reflected in behaviour. In 1983 (Figure 19), neither time nor treatment differences logically separate the groups, and the interpretation of the spatial separation of the groups is hightly speculative. One reason might by that the fly pressure in 1983 was very low. Without a significant fly pressure, behavioural responses and treatment differences are not easily distinguished.

CONCLUSIONS

Mosquitoes, horse flies and stable flies caused an increase of individual behavioural responses such as head tosses, foot stomps, tail switches and ear flicks. However, the greatest impact of biting flies on the heifers (especially the horse flies) was their effect on group behaviour. During attacks by horse flies, animals would graze in a linear formation, or during severe attacks, would stop grazing and form tight bunches. The animals were tolerant of feeding by mosquitoes, except for one observation in June, 1983 when the animals bunched and stampeded. Similarly, the animals were tolerant of feeding by stable flies. There were no significant differences of behavioural responses (head tosses, foot stomps, tail switches and ear flicks) due to treatments. This may be because cypermethrin has no apparent repellency. Thus, flies were attracted to, and attacked the treated animals with the same result as the control animals. Even if cypermethrin is an effective insecticide (as is indicated by the bioassay, Manuscript 1) this effectiveness happens after feeding or harassment has taken place. Results from the 24-hour observations of behaviour are inconclusive. If the 28 June observation had been taken during the peak of the horse fly population, differences between the dates might have been significant. The only indication from this study that treatment made a significant difference in behavioural

responses, was in the second half of the 1984 fly season. Although the data are only from one field season, the implications are that treatment differences are significant when populations of horse flies are at a peak. Treatment of pastured animals may not be necessary when populations of horse flies are low. However, these significant treatment differences are in behaviour and were not reflected in significant weight gain differences. Thus, the cost of treatment may not be regained by increased average daily gains.

The results of this study reflect the summer environmental conditions of 1983 and 1984 for Seven Sisters, Manitoba. They indicate trends which might occur in other areas under similar conditions. Whether the impact of biting flies on pastured cattle is a direct result of blood feeding, harassment or thermoregulation of the animals, is unknown. Control of biting flies is becoming increasingly costly, and in areas such as Seven Sisters, larviciding is impractical. Perhaps more care should be taken in the decison of converting marginal land, with poor drainage, into pastureland.

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APPENDICES

Appendix A. The location of origin, date of birth, and initial weight of the heifers in 1983. All animals were Holsteins unless otherwise noted.

Location of Producer	Tag #	Date Born	Initial Weight (Kg)
Whitemouth	72	12 April 1982	283
Whitemouth	73	6 April 1982	248
Whitemouth	74	13 April 1982	283
Whitemouth	75	28 May 1982	239
La Broquerie	76	------	247
La Broquerie	77	------	233
La Broquerie	78	3 July 1982	273
La Broquerie	79	22 June 1982	319
La Broquerie	80	March 1982	352
La Broquerie	81	May 1982	308
La Broquerie	82	July 1982	292
La Broquerie	83	July 1982	293
St. Pierre	84	------	336
St. Pierre	85	------	364
Whitemouth	86	Sept 1982	267
Whitemouth	87	Sept 1982	263
Whitemouth	88	Sept 1982	234
Whitemouth	89	Sept 1982	215
Whitemouth	90	Apr 1982	342
Whitemouth	91	May 1982	275
Whitemouth	92	May 1982	317
Whitemouth	93	May 1982	321
Steinbach	94	12 April 1982	230
Steinbach	95	8 April 1982	235
Steinbach	96	15 March 1982	268
Steinbach	97	25 March 1982	230

continued.....

Appendix A. (cont.)

Location of Producer	Tag #	Date Born	Initial Weight (Kg)
Beausejour	98	2 March 1982	261
Beausejour	99	8 May 1982	226
Beausejour	100	3 March 1982	265
Beausejour	39	14 March 1982	245
Beausejour	77R	------	290
Beausejour	78R	------	266
Beausejour	75R	------	356
Beausejour	76R	------	275
Beausejour	71R	------	232
Beausejour	72R	------	386
Beausejour	73R	------	351
Beausejour	74R	------	334
Lorette	15	Oct 1982	212
Lorette	11	Oct 1982	234

Appendix B. The location of origin, date of birth, and initial weight of the heifers in 1984. All animals were Holsteins unless otherwise noted.

Location of Producer	Tag#	Date Born	Initial Weight (Kg)
La Broquerie	13R	11 June 1983	260
La Broquerie	1	29 Aug 1983	245
La Broquerie	2	18 Sept 1983	238
La Broquerie	3	29 Aug 1983	224
Beausejour	14	------	256
Beausejour	7	------	268
Beausejour	6	------	266
Beausejour	11	------	285
Beausejour	15	------	276
Beausejour	8	------	254
Beausejour	12	------	224
Beausejour	13	------	232
Lorette	9	27 Aug 1983	234
Lorette	10	5 Sept 1983	232
Lorette	4	------	304
Lorette	5	------	312
Tyndall	16	4 March 1983	274
Tyndall	17	25 March 1983	258
Tyndall	18	2 Feb 1983	296
Tyndall	19	11 April 1983	236
Whitemouth	21	8 April 1983	241
Whitemouth	20	4 April 1983	265
Whitemouth	22	1 March 1983	266
Whitemouth	23	17 Feb 1983	281

continued.....

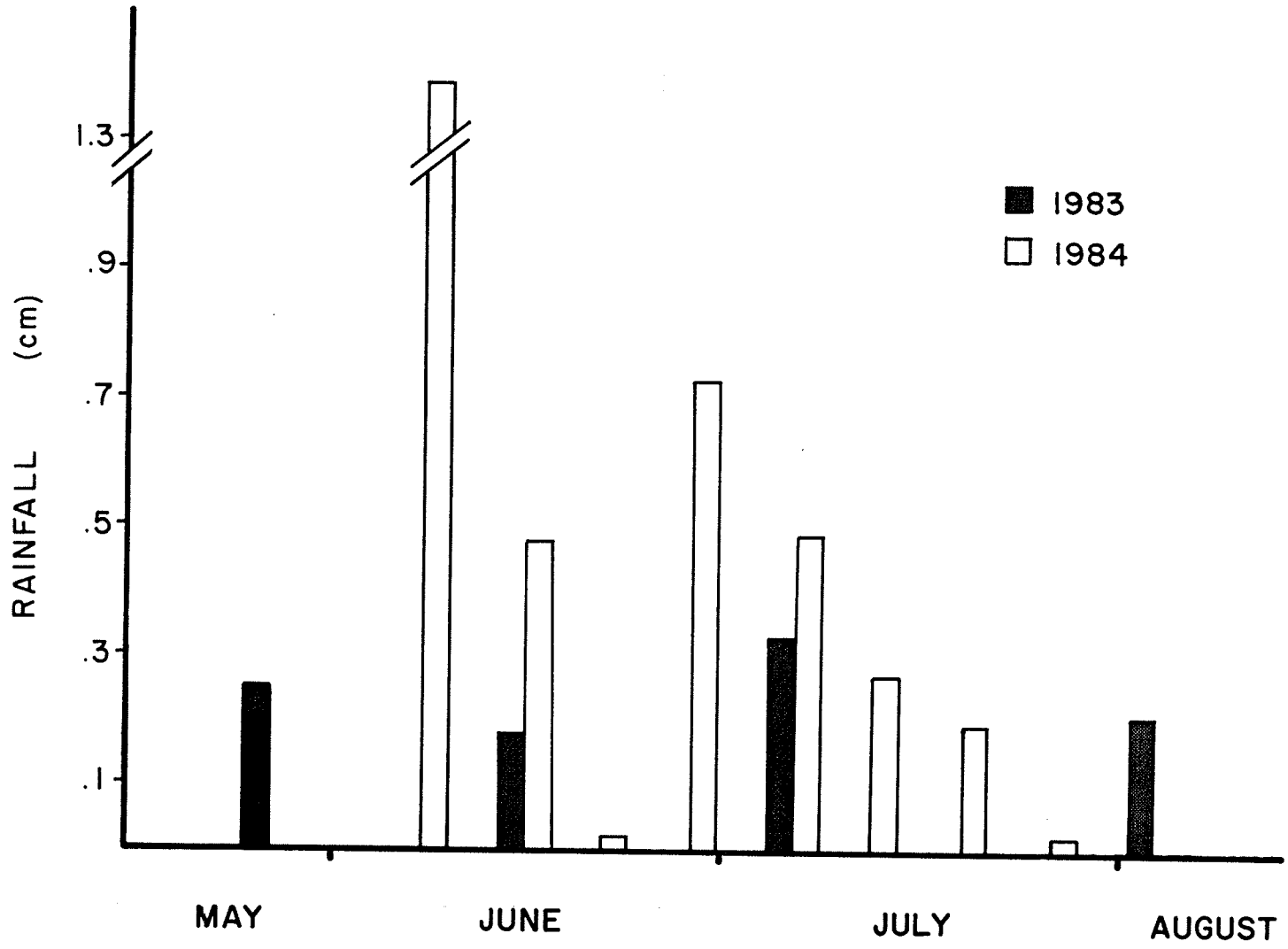
Appendix B. (cont.)

Location of Producer	Tag#	Date Born	Initial Weight (Kg)
Whitemouth	24	1 June 1983	324
Whitemouth	25	25 June 1983	320
Whitemouth	26	2 June 1983	310
Whitemouth	27	22 June 1983	326
East Selkirk	28[a]	4 May 1983	153
East Selkirk	29[a]	March 1983	211
River Hills	66R[b]	15 Oct 1983	182
River Hills	97R[b]	14 Sept 1983	241
River Hills	74R[b]	15 Oct 1983	229
River Hills	74R[b]	12 Oct 1983	188

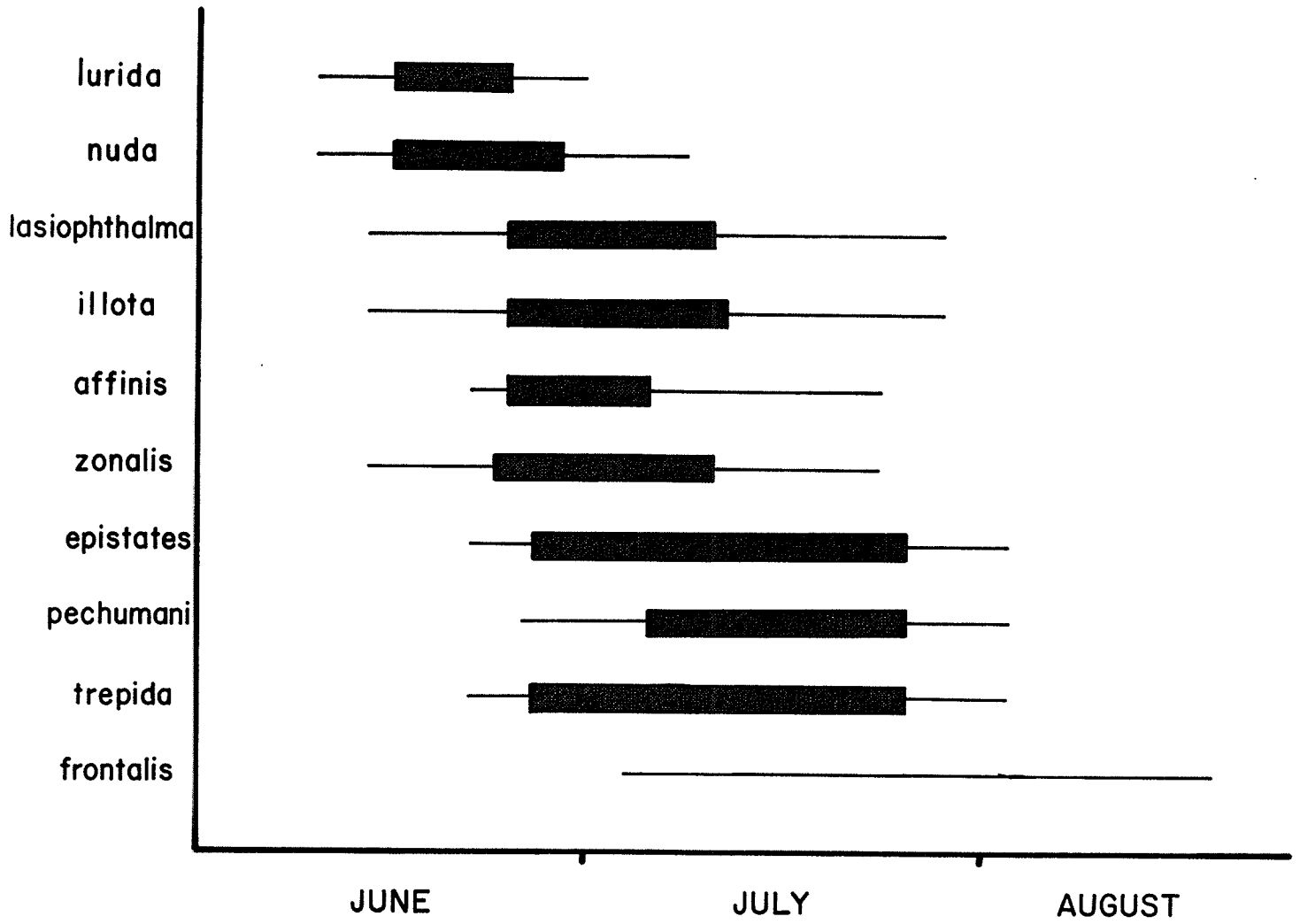
[a] Jersey x Brown Swiss

[b] Jersey

Appendix C. The weekly averages of rainfall (in cm) at Seven Sisters, Manitoba, 1983 and 1984.


RAINFALL (cm)
1.3
.9
.7
.5
.3
.1
1983
1984
MAY
JUNE
JULY
AUGUST

Appendix D. A graphic representation of the common species of Hybomitra at Whitemouth Lake, Manitoba, 1979. The line represents the 1st and last date that the species were collected in the MHFT, and the solid bar represents 75% of the trapped population.


lurida
nuda
lasiophthalma
illota
affinis
zonalis
epistates
pechumani
trepida
frontalis
JUNE
JULY
AUGUST

Appendix E. An explanation of Discriminant Analysis.

The purpose of this appendix is to illustrate by way of a step-wise example, the procedure of canonical discriminant analysis. The data used in this explanation have been taken from the original data set of the 1984 daily counts. Twenty observations from each of the control and treated herds were selected at random. These two data sets are compared without the consideration of time, as with the original data set. Two variables (tail switches and foot stomps) have been chosen to simplify the explanation of the analysis. Interpretation of the results using 2 variables is different from an analysis using 4 variables, and thus conclusions drawn from this example may not be the same as those for the complete data set. Where possible, diagrams are used to facilitate the explanation of the analysis.

ANALYSIS

Univariate and Bivariate. Univariate statistics are concerned with the distribution and variation of a single dependent variable. Bivariate statistics compare one variable to another and their bivariate plot considers the joint distribution of their covariation. Conclusions based upon univariate analysis can differ from those based on bivariate analysis. In the case where multiple variables are used to describe a number of data sets, the complexities of covariation must be analyzed using multivariate methods.

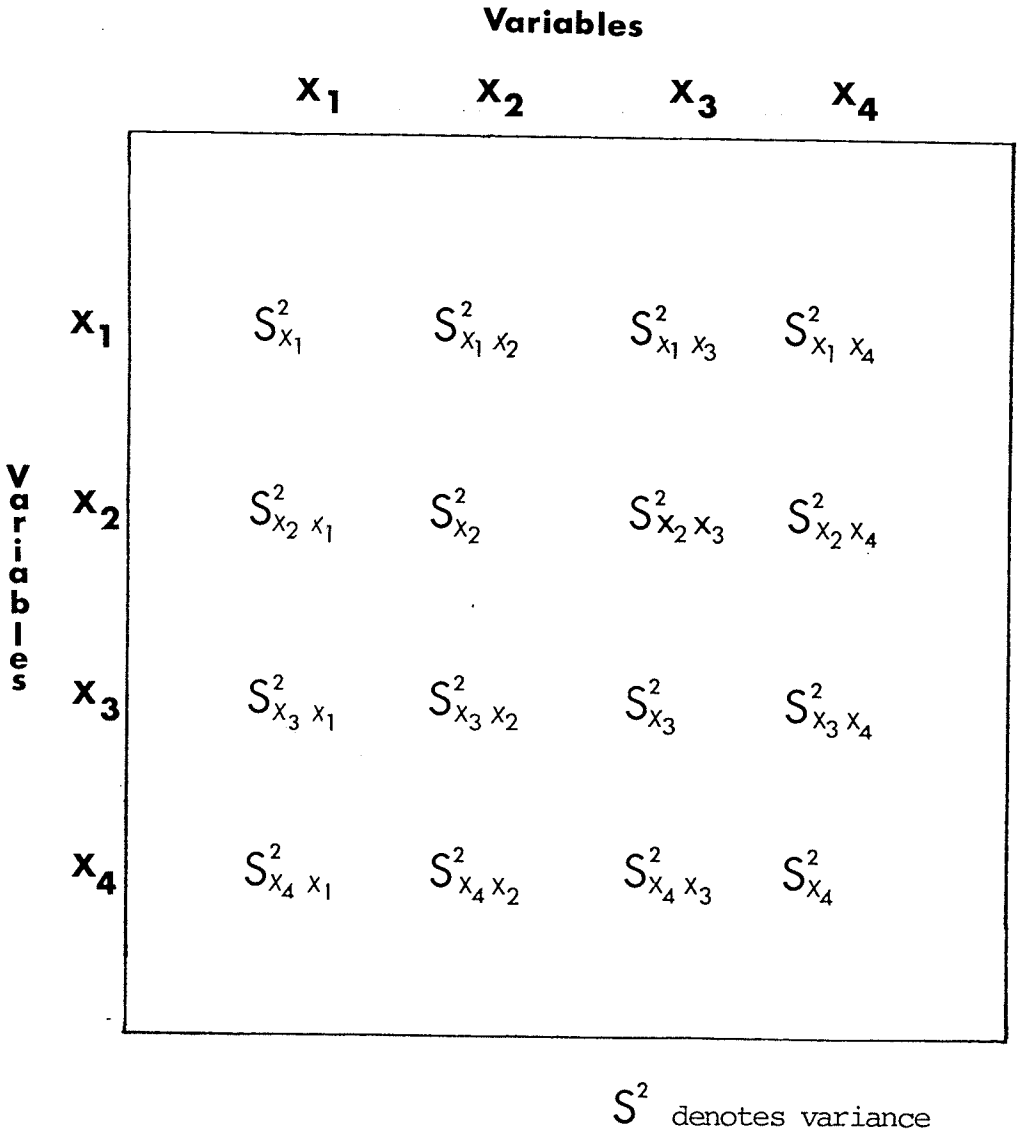
Multivariate. As with univariate analysis, multivariate analysis is used for the analysis of all sources of variation of the variables. However, multivariate analysis offers the advantage of simultaneously considering the joint distribution of all variables. One particular type of multivariate analysis, discriminant analysis, can be used to investigate the similarities or differences between groups. The term 'group' is defined as a biologically distinct unit. The total variablility of observations in this type of analysis is partitioned into:

1. The variability related to the distribution of individual data points around their own group mean (or centroid) or the within-group variability, and
2. The variability related to the overall distribution of the group means, or the between-group variability.

It is these sources of variation for all variables which are considered simultaneously.

The major assumption of discriminant analysis is equality of the covariance matrices of the data sets or groups. Algebraically, the covariance matrix is the symmetrical table of the covariances of each variable in a set of variables compared with every other one (see Pielou 1984, page 103). The diagonal of this matrix is the variance of each variable (Table A1). If the assumption of equality is not met, the test is robust enough to use if the data set is

Table A1. A covariance matrix in which the rows and columns comprise of the covariances of one independent variable with every other independent variable and the diagonal of the matrix is the variance of the independent variable.



large and equal. Just how large the data set must be is speculative, however 25 individuals per group is considered adequate by some authors (e.g., Pimentel and Frey 1978). Canonical discriminant analysis may be employed as a means of exploratory data analysis and, as such, distributional assumptions of the data are not required (Gittins 1979). If the data are analyzed as such, the presence of certain relationships or effects can be determined.

Canonical discriminant analysis involves 3 steps as outlined by Pimentel and Frey (1979), and each step reveals information about group differences.

1) The first step is to test the equality of multivariate group means (or centroids) by way of a multivariate analysis of variance (MANOVA). The MANOVA also tests for homogeneity of the group covariance matricies (the major assumption of the test). The purpose of this step is to determine whether differences exist between populations (e.g., treated and control groups) before studying the nature of these differences.

2) The second step of discriminant analysis is to classify each observation into either its own group or another. This involves the square of the Mahalanobis distance of each observation from its group centroid (see Pimentel and Frey 1978). (The Mahlanobis distance is the distance between the means, taking into account the

within-group covariance.) Thus a decision is made in assigning an observation to one of the groups in the analysis. The probability of the group means being greater than the Mahalanobis distance is an indication of which groups are significantly different. This step gives an indication of how unique, or different, each group is from the other, and the observations are used as a measurement of this difference (Pimentel and Frey 1978).

3) The last step in discriminant analysis (in this case specifically canonical discriminant analysis) is analysis of the relationships between the groups in 'canonical discriminant space'. The greatest statistical separation among the groups is defined (Albrecht 1980). The coordinate frames or axes of the original data are rotated to a new position in which the covariance between the 2 sets of variables is maximized (Gittins 1979). The series of rotations and standarizations is based upon the within-group and between-group variations. The transformed coordinate axes no longer represent the original variables, but rather the resulting axes, or canonical variates, represent some special qualities or variables which separate the groups. The canonical variable 1, which represents the horizontal axis, emphasizes the major axis of between-group variation. It is selected so that it represents the greatest possible difference between groups. Each successive canonical variate is selected by the same criterion (between-group

variance) and is orthogonal to all preceding axes. Vectors of the original variables can then be plotted on the canonical graph to indicate the influence of those variables in that space (Jolicoeur 1959). These vectors begin at the origin of the canonical axes (the grand centroid) and are plotted according to the coefficients of the variables. Each vector indicates the positive 'push' for that variable (Pimentel and Frey 1978). A group mean which extends just beyond a long vector representing a particular variable, identifies that group as having a large number of measurements for that variable. Similarly, a group mean which occurs in the opposite direction to the vector represents a low number of measurements of that variable in that group. Further interpretation of the vectors may indicate how the rotation affected the original variables, and which variables tend to interact versus those which are antagonistic (Pimentel and Frey 1978).

The literature concerning discriminant analysis is vast and much of this published work involves computational methods. Historically the usage of discriminant analysis has been limited to recent years by the advantage of computers for the large number of calculations. In the biological sciences, discriminant analysis has been used to study the nature of group differences in taxonomy, morphometrics and community structures. Discriminant analysis is probably best known in numerical taxonomy (Sokal

and Sneath 1973). Simply, it is the numerical analysis of similarities between groups of individuals and the subsequent ordering of these groups into different taxa. Similarly, morphometrics, or the numerical study of biological form, involves problems in which multidimentional measurements are taken from individuals and used to describe relationships among populations (Albrecht 1980; Jolicoeur 1959). Although the use of multivariate techniques is minimal in behavioural research, its usage is becoming more acceptable (Pimentel and Frey 1978). Recently multivariate methods are being used to analyze group differences based on behavioural variables (Frey and Pimentel 1978).

EXAMPLE

In the following example, each step of discriminant analysis will be explained using real data and graphs where possible. As mentioned earlier, the data set is taken from the complete data set of 1984 using lfoot and ltail as variables. However, the data for this example have been modified to emphasize each step of the procedure. The data were collected as outlined in the Materials and Methods section of Manuscript 2. The two groups being compared are

Group 1 = control;

Group 2 = treated.

As with the entire data set, the observations in this example have been transformed (natural logarithm) to meet the assumption of homogeneity. These variables will be hereafter referred to as foot and tail.

The univariate statistics for tail and foot are summarized in Table A2. There is no significant difference (P=.05) between the treated and control animals with respect to foot stomps. However, there is a significant treatment effect of tail switching. This univariate test considers variation due to treatments for a single variable at a time. In Figure A1, the axes of foot and tail represent the univariate distribution for each variable. The axis of foot includes the distribution of Group 1 and Group 2 for that variable (foot). Similarly, the vertical axis contains the distribution of Groups 1 and 2 for the variable tail. In univariate statistics, the distribution of each variable along the axis must be observed separately, and complete separation of the 2 groups is not evident when the 2 variables are considered separately (see Green 1979).

Results of first step in discriminant analysis (MANOVA), indicates there there is a significant difference between the group centroids (F=31.68 with 2 and 37 df, P=.0001); i.e., between the treated and control herds.

The classification of the variables tail and foot into either Group 1 or 2 is summarized in Table A3. Ninety-five

Table A2. The analysis of variance for treatment differences for the variables tail and foot.

Variable tail

Source of variation	df	SS	MS	F	
treatments	1	67.66	67.66	59.52	*
error	38	43.20	1.137		

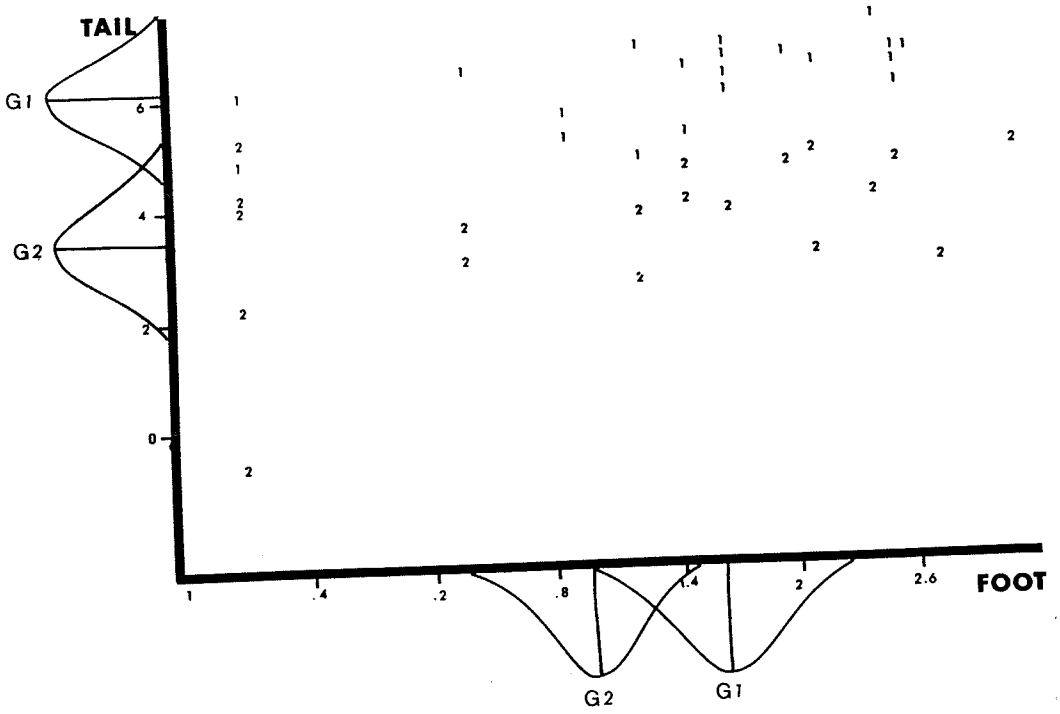
Variable foot

Source of variation	df	SS	MS	F	
treatments	1	2.013	2.013	1.464	ns
error	38	52.25	1.375		

ns denotes non-significance
* denotes significance ($\alpha \leq .05$)

Figure A1. (a) A plot of the original data with the numbered points representing Group 1 or Group 2. The univariate graphs for foot and tail are represented by normal curves and group means along each axis.
(b) Group means (treatment means) for tail and foot.

a



b

Treatment Means (Group) for TAIL and FOOT

	TAIL	FOOT
Group 1	6.139	1.498
Group 2	3.537	1.049

Table A3. Classification of the raw data (tail and foot) into either Group 1 or Group 2.

	Number of Observations and Percentages Classified into Group:		
	1	2	Total
From Group: 1	19 95.00%	1 5.00%	20 100.00%
From Group: 2	1 5.00%	19 95.00%	20 100.00%
Total Percent	20 100.00%	20 100.00%	40 100.00%

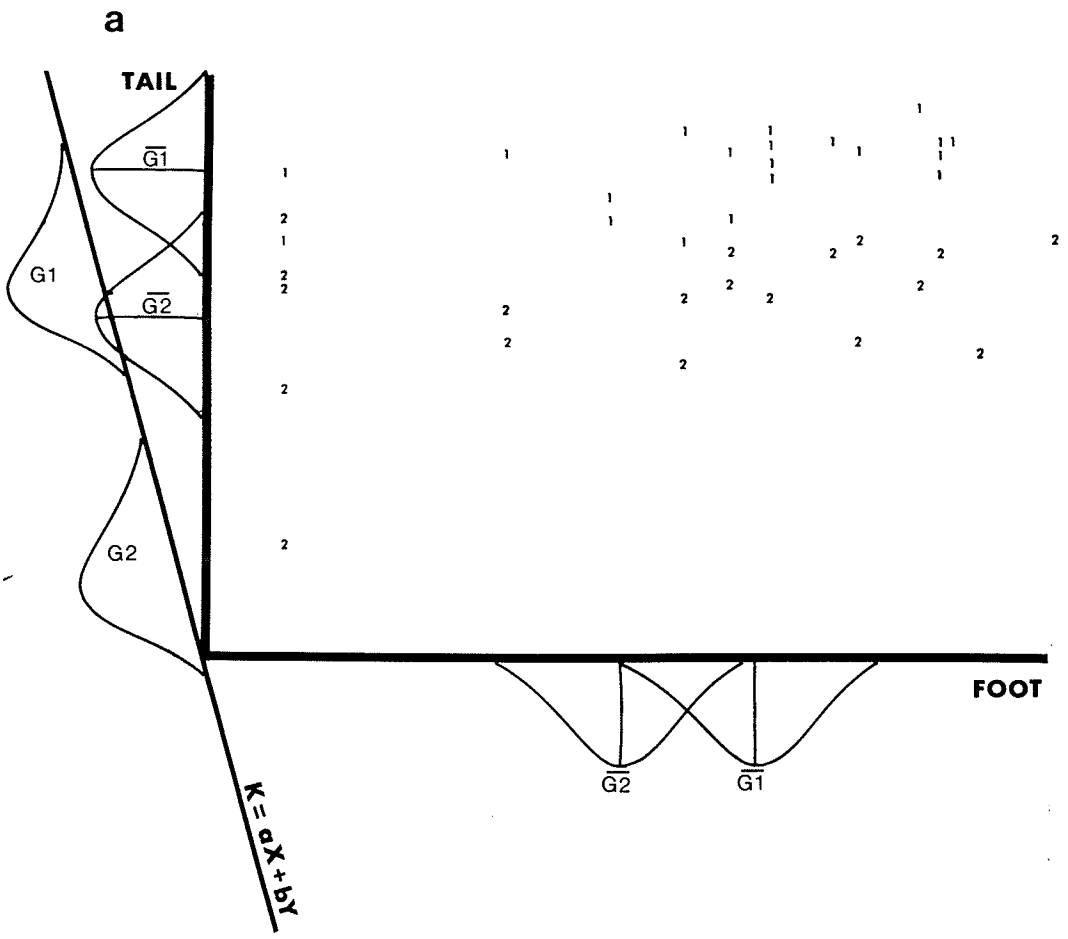
percent of the tail and foot observations have been correctly classified into their own groups by this step of the analysis.

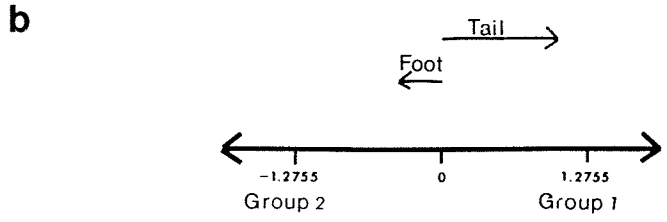
The last step in canonical discriminant analysis is the analysis of group relationships in canonical space. From the original graph (Figure A1), the distributions of tail and foot are not easily distinquishable. However the line K=aX+bY corresponds to the projections of distributions for Groups 1 and 2 showing their maximum separations. This new variable, K, is the "discriminant function", and describes the vector exposing maximum discrimination of the groups. This can be seen by the distributions along the discriminant function K (Figure A2). The possible number of discriminant functions is equal to one less than the number of groups, and is equal to the number of unique variables which separates the groups (Green 1979).

With 2 groups, there is only one discriminant function or one major variable which is unique to each group and in this example, the variable is treatment. This variable is also called the "canonical variable" and it is the factor or variable in each group which is intrinsic, or unique. This canonical variate is derived from a series of rotations of the original coordinate axis (tail and foot) to a new position which emphasizes group differences based upon the canonical variate. All variation due to within- and between-group differences is revealed in discriminant space.

Figure A2. (a) A graph of the original data points and normal plots, however, the distribution of the groups is now represented on the discriminant function $K=aX+bY$, along which maximum separation of the groups is observed.

(b) The group means are represented on the axis of the canonical variate and the distance which separates them is the Mahalanobis distance. The vectors of foot and tail represent the canonical correlation coefficient.


a
TAIL
G1
G2
G1
G2
FOOT
G2
G1
K = aX + bY


b
Tail
Foot
-1.2755
0
1.2755
Group 2
Group 1

The correlation of the canonical variates is called the "canonical correlation coefficient". This coefficient is the amount of weighting or the index which is applied to each observation of the original data. Thus, from the first observation of Table A4, each canonical variable (CAN 1) is derived from multiplying the coefficient by the difference between the observation and the variable mean. CAN 1 = CCCF ($\bar{x}$-lfoot) + CCCT ($\bar{x}$- ltail)

CAN 1 = -0.2764(2.525-1.2374)+1.0283(6.49424-4.8382)

=1.3570

CCCF - canonical correlation coefficient of lfoot

CCCT - canonical correlation coefficient of ltail

$\bar{x}$F - mean of lfoot

$\bar{x}$T - mean of ltail

Since there is only one canonical variate, the axis can be represented on Figure A3(b). The distance between the group means is the Mahalanobis distance, and in this example the Mahalanobis distance is 2.5510. The coefficients for tail and foot are represented by vectors from the grand centroid (zero) and indicate the direction of influence for that variable.

If there were 3 groups in this example rather than 2, the hypothetical scatter diagram of foot and tail might look like Figure A3(a). The nature and magnitude of the

Table A4. A tabular summary of the data set including the means of the transformed variables, canonical coefficients, and the canonical variate.

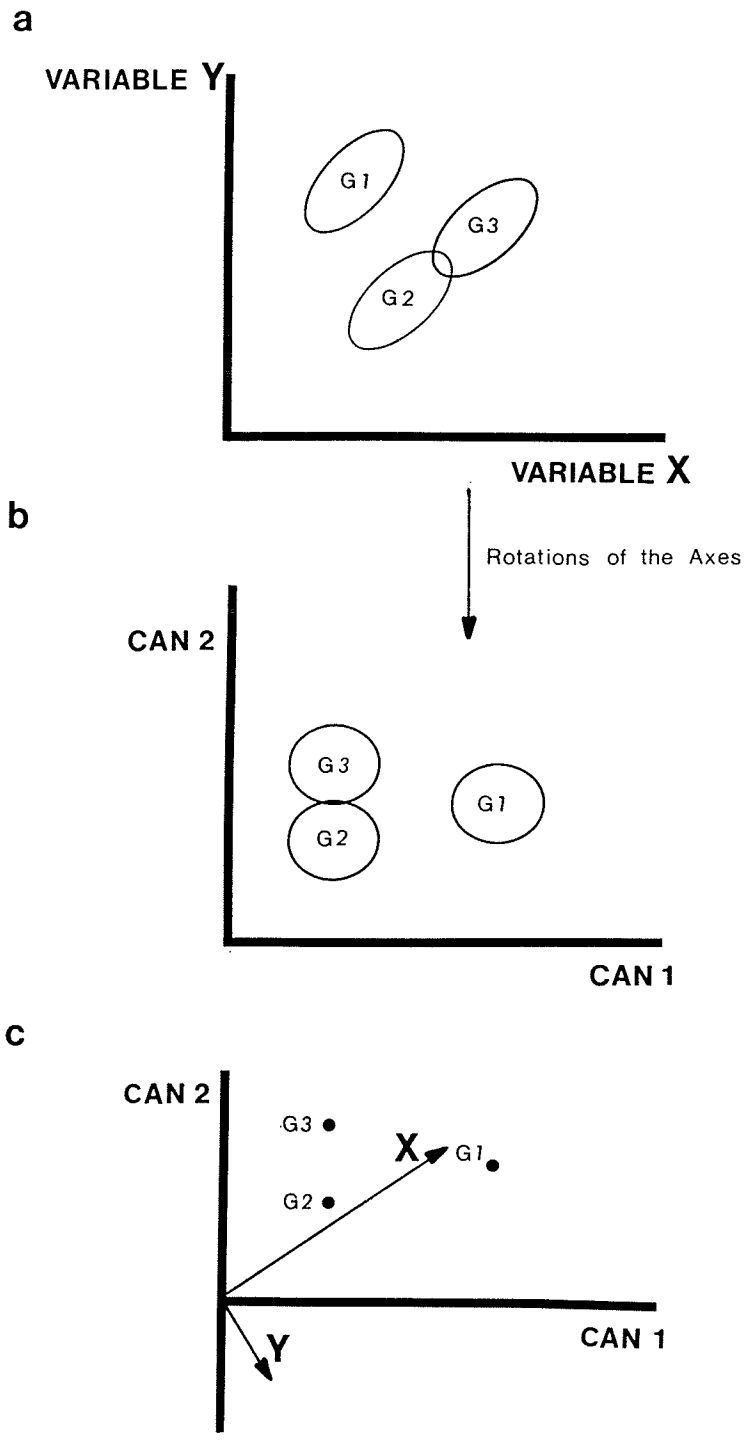
Observation	Original Foot	Original Tail	Transformed Lfoot	Transformed Ltail	Group	CAN 1
1	12	89	2.52573	6.49424	1	1.3570
2	3	120	1.25276	6.79165	1	2.0146
3	3	15	1.25276	4.74084	1	-0.0943
4	0	52	-.069315	5.96081	1	1.6980
5	5	107	1.70475	6.67749	1	1.7723
6	2	24	0.91629	5.19867	1	0.4695
7	2	36	0.91629	5.59731	1	0.8794
8	5	57	1.70475	6.05178	1	1.1289
9	12	95	2.52573	6.55913	1	1.4237
10	4	25	1.50408	5.23868	1	0.3484
11	0	15	-0.69315	4.74084	1	0.4435
12	12	53	2.52573	5.97968	1	0.8278
13	5	120	1.70475	6.79165	1	1.8897
14	7	100	2.01490	6.61016	1	1.6174
15	1	89	0.40547	6.49424	1	1.9430
16	5	72	1.70475	6.28359	1	1.3673
17	11	187	2.44235	7.23378	1	2.1405
18	13	95	2.60269	6.55913	1	1.4025
19	4	85	1.50408	6.44852	1	1.5923
20	8	75	2.14007	6.32413	1	1.2886
21	3	13	1.25276	2.60269	2	-2.2931
22	3	44	1.25276	3.79549	2	-1.0665
23	11	51	2.44235	3.94158	2	-1.2450
24	15	15	2.74084	2.74084	2	-2.5623
25	5	41	1.70475	3.72569	2	-1.2632
26	0	0	-0.69315	-0.69315	2	-5.1446
27	1	38	0.40547	3.65066	2	-0.9813
28	4	108	1.50408	4.68675	2	-0.2194
29	12	96	2.52573	4.56954	2	-0.6223
30	22	133	3.11352	4.89410	2	-0.4510
31	0	8	-0.69315	2.14007	2	-2.2311
32	0	49	-0.69315	3.90197	2	-0.4192
33	0	66	-0.69315	3.19720	2	-0.1156
34	7	94	2.01490	4.54860	2	-0.5026
35	8	115	2.14007	4.74927	2	-0.3309
36	0	173	-0.69315	5.15618	2	0.8706
37	0	9	-0.69315	2.25129	2	-2.1167
38	1	19	0.40547	2.97041	2	-1.6806
39	8	20	2.14007	3.02042	2	-2.1087
40	4	49	1.50408	3.90197	2	-2.0264

X_F=1.27374 X_T=4.83819

Canonical Correlation Coefficients (CCC)

Foot = -0.2764
Tail = 1.0283

Figure A3. Graph of the hypothetical variables X and Y involving 3 groups, (a) the bivariate graph of variable X on variable Y and the dispersion of the data points for the 3 graphs represented by ellipses, (b) graph of the canonical variables after standardization of the data and rotation of the axes, (c) vectors of the original variables (X and Y) plotted on the canonical axes with the dots representing the group means.


a
VARIABLE Y
G1
G3
G2
VARIABLE X
b
Rotations of the Axes
CAN 2
G3
G2
G1
CAN 1
c
CAN 2
G3
X
G1
G2
Y
CAN 1

differences between groups does not show up well when the variables are considered one by one (Jolicoeur 1959). After each source of variation (within-group and between-group) has been analyzed and standardized, the final canonical graph might look like Figure A3(b). The relative position of the groups and thus the between-group variation, is maximized.

Now we have 2 major discriminant functions, or lines which show maximum variation. The canonical variate 1, or horizontal axis, depicts the primary source of between-group variation, and is orthogonal to the 2nd. Vectors of the original data, e.g., ltail and lfoot, can be plotted on the canonical graph (Figure A3(c)) from the coefficients of the canonical variables. These vectors may indicate which group has a large number of measurements of the original variable (lfoot and ltail).

INTERPRETATION

Each step of discriminant analysis reveals information about the differences and similarities of the groups being studied. The MANOVA simply reveals that indeed there are significant group differences. However, information about which group is different from another is not given at this step. The classification step assigns an observation to the group it most closely approximates in form (Pimentel and Frey 1978). This step is based on the fact that the actual

group to which each observation belongs, is known. This step also reveals information about which group is significantly different from the others, based upon the Mahalanobis distance. In this example, 95% of the classification was correct, and there is a unique difference between Group 1 and Group 2.

Interpretation of the final step is based upon graphically observing direct group differences. It is important at this stage to understand the nature of group differences. The canonical variates represent some unique property or variable which is present in each group. These variables do not have to be directly measured and can represent for example, some ecological or environmental characteristic. In this example, treatment is most likely the factor which significantly separates Group 1 from Group 2. Thus, it would seem a likely conclusion that treated animals behave differently from control animals based upon foot and tail behaviour responses. Further, Group 1 or the control animals, have a higher number of tail switches than the treated animals. The spatial separation of Group 1 from Group 2 is primarily a result of the number of tail switches. The magnitude of 'foot' is smaller than 'tail', but is also partially responsible for the spatial separation of the groups. Since the number of tail switches is higher in the controls, this may be in part caused by the cypermethrin spray. The hypothetical vectors in Figure

A3(c) can be interpreted as vector 'X' being responsible for the horizontal separation of Group 1 from Groups 2 and 3. Vector 'Y' is also responsible for some of the horizontal separation, but with less magnitude.

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