Horn Fly Susceptibility to Diazinon, Fenthion, and Permethrin at Selected Elevations in Wyoming[1]

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ABSTRACT Horn fly, *Haematobia irritans* (L.) (Diptera: Muscidae), populations from southeastern Wyoming were assayed for resistance against permethrin, diazinon, and fenthion. All of the populations examined were significantly more resistant to permethrin than a susceptible strain. Eight of 25 populations examined were significantly more resistant to diazinon than a susceptible strain. Five of 15 populations examined were significantly more resistant to fenthion than a susceptible strain. No relationship was detected between elevation and resistance levels.

KEY WORDS *Haematobia irritans*, Diptera, Muscidae, horn fly, resistance, elevation, bioassay

The horn fly, *Haematobia irritans* (L.) (Diptera: Muscidae), is one of the most important insect pests of beef cattle in the United States (Bruce 1964). Its blood-feeding habits and its occurrence in large populations can cause decreased weight gains, feed efficiency, and milk production (Campbell 1976; Kinzer et al. 1984; Kunz et al. 1984; Cocke et al. 1989).

Horn flies overwinter as diapausing pupae and emerge as adults the following spring. Horn fly pupae enter diapause in the fall, and the proportion of pupae in diapause increases as temperatures decline (Lysyk & Moon 1994). Timing of diapause completion and adult eclosion are temperature dependent, and occur earlier in the spring at higher temperatures (Miller 1977; Miller & Kunz 1985). Emergence of adult horn flies occurs over a 20–40-d period (Bruce 1964, Hoelscher & Combs 1971). Mating and oviposition begin soon after adult emergence, and immature developmental time can vary from 14 to 25 d (Lysyk 1992). Because of the variation in adult emergence and immature developmental time, horn fly generations overlap and emergence is continuous throughout the fly season.

Ear tags containing pyrethroid and organophosphate insecticides have been used widely for the control of horn flies since 1980. However, development of resistance in horn flies exposed to insecticides used in ear tags has been reported in many areas of the United States (Sheppard 1983, 1984; Harvey et al. 1984; Quisenberry et al. 1984; Kunz & Schmidt 1985; Schmidt et al. 1985; Cilek &

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Knapp 1990; Cilek et al. 1991; Sheppard & Joyce 1992). These early cases of resistance occurred in areas of multiple generations per season, long fly seasons, and high numbers of flies on cattle.

Many methods for testing horn fly resistance to insecticides have been examined, with varying results. These tests can be categorized into two groups: direct application, including topical and feeding methods (Harris 1964, Sheppard 1983) or residual assays that include petri dish, glass test tube, treated cloth, and filter paper techniques (Sheppard 1984; Schmidt et al. 1985; Cilek & Knapp 1986; Sheppard & Hinkle 1986, 1987). Residual assays have become the method of choice due to ease of use and ability to test larger numbers of flies more quickly. Herds of cattle in Wyoming occur in extremes of elevation, from 1,250 to >3,000 m. Wyoming, unlike the other areas with known horn fly resistance, has a short horn fly season with few generations per season. Because horn fly densities and control practices appear to be different at extremes of elevation, insecticide resistance levels also might be expected to differ. For example, if densities of horn flies are lower at higher elevations, a producer may be less likely to use prophylactic insecticide treatments. The status of horn fly pyrethroid and organophosphate resistance in Wyoming and the influence of elevation on selection for resistance is unknown. The objective of this study was to determine the status of horn fly resistance in Wyoming to the pyrethroid insecticide permethrin, and to the organophosphate insecticides diazinon and fenthion, and to determine whether elevation had an influence on resistance.

Materials and Methods

Cattle ranches within 160 km (2.5-h drive) of Laramie, Wyoming, were identified by university extension agents, animal scientists, and cattle producers. Ranch selection was not made based on treatment history; it was by invitation of producers. To determine the relationship between resistance levels and past use of insecticides, producers were questioned about prior insecticide use.

During 1995, adult horn flies from 12 Wyoming ranches were assayed for susceptibility to diazinon and permethrin. In 1996, adult horn flies from 14 Wyoming ranches were assayed for susceptibility to permethrin and fenthion, and flies from 12 ranches were evaluated for diazinon susceptibility. Flies from one herd in Nebraska also were assayed for susceptibility to diazinon and fenthion in 1996. Adult horn flies were collected from 11 August 1995 to 17 September 1995 and from 9 July 1996 to 13 September 1996, when, in each year, freezing temperatures in September severely reduced horn fly populations, ending the study.

For comparison, horn flies from two laboratory colonies from the USDA–ARS U.S. Livestock Insects Research Laboratory, Kerrville, Texas, were included in the study. The susceptible colony was established with flies collected near Kerrville ≈30 yr ago. The flies had never been exposed to insecticides. A pyrethroid-resistant laboratory colony was established in August 1992 by collecting cyhalothrin-resistant flies. Cyhalothrin resistance has been maintained in this colony, the USDA–ARS Kerrville cyhalothrin-resistant strain, by exposing flies to a bovine host treated weekly with topical applications of cyhalothrin, 24 at 260 μg (AI)/ml.

Two assays, topical application and treated filter paper, were used to determine the level of insecticide resistance of horn flies. The treated filter paper bioassay developed by Heim et al. (1990) used formulated insecticides and was modified to determine dose-mortality slopes for horn fly populations. The permethrin formulations Atroban Swine and Mange EC®, 11% (AI), used in 1995 and 1996, and Atroban 11% EC®, 11% (AI), used in 1996, were supplied by Mallinckrodt Veterinary, Mundelein, Illinois. The diazinon formulation Diazinon 4E®, 47.5% (AI) was supplied by Ciba Corporation, Greensboro, North Carolina. The fenthion formulation Lysoff®, 8% (AI), was supplied by Bayer Corporation, Shawnee Mission, Kansas.

Serial dilutions of these five insecticides were made in acetone. One milliliter of each concentration was applied to a labeled 9-cm Fisherbrand P5 filter paper disk (Fisher Scientific, Pittsburgh, Pennsylvania) that was then placed on crinkled aluminum foil to facilitate drying with minimal contact loss of insecticide. All treated filter paper disks were cured at least 24 h before each assay (Sheppard & Hinkle 1987). After drying, all treated and control (acetone only) filter paper disks were placed by dose in sealed plastic bags and stored at −15°C.

Adult horn flies were collected from the backs of cattle by using an aerial insect net. Flies were placed in a 13 by 18 by 30 cm screened cage that was then placed in a self-chilling cooler at 7° or 8°C for transport to the laboratory. In the laboratory, flies were held at 4°C for 10 to 30 min until they were placed on the treated filter papers.

Treated filter papers were placed in the lids of 100 by 15 mm disposable polystyrene petri dishes (one filter paper per lid). One milliliter of distilled water was placed on each filter paper. All dosages and an acetone-only control were replicated four times. Flies were anaesthetized with CO_2 and placed on a cold plate (1.7°C) until transferred to petri dishes in groups of from 10–30 flies. Following loading of all dishes with flies, ≈5 to 10 min, the flies were examined for mortality and the number of dead flies was recorded. Following pretrial observations of flies resting on the untreated lids, petri dishes were inverted so the filter paper was positioned above the flies to maximize contact. Results were recorded after 2 h of exposure. Any fly unable to walk at that time was considered dead. All assays were run between 0900 and 1700 hours (MT) under constant fluorescent light emitted from ceiling fixtures, and at a room temperature of ≈20°C.

The topical bioassays were conducted using a Model M Microapplicator (Instrumentation Specialties, Lincoln, Nebraska) that applied 0.4 μl of serial dilutions of formulated permethrin in acetone to the thorax of chilled, CO_2 anaesthetized adult flies. Following insecticide application, 10 to 25 treated flies were placed in a 120-ml wax-coated cup with an inverted bottom of a plastic petri dish serving as a lid. Results were recorded after 2 h of exposure, and any fly unable to walk was considered dead. All assays were run between 0900 and 1400 hours under lighting and temperature conditions described previously. Insecticide dilutions were selected to provide between 5 and 95% mortality at a minimum of five dosages for each population. The initial five dosages were determined with horn flies from the susceptible laboratory colony for each of the insecticides (Table 1). Dosages for each Wyoming population and the USDA–ARS Kerrville cyhalothrin-resistant strain were incrementally increased until the mortality was between 5 and 95%.

Probit analysis was conducted on all data by using POLO-PC (LeOra Software 1987). Probit regression lines were estimated individually for each population and insecticide. All hypotheses were tested by the likelihood ratio test (Savin et al.

Table 1. Five insecticide dosages that provided between 5 and 95% mortality of the USDA—ARS Kerrville-susceptible horn fly strain, over 2 yr.

Year 1 (1995)[a]		Year 2 (1996)			
Disc permethrin	Disc diazinon	Disc[a] permethrin	Topical[b] permethrin	Disc[a] diazinon	Disc[a] fenthion
0.00356	0.00830	0.00356	0.000091	0.0160	0.0099
0.00405	0.01810	0.00405	0.000103	0.0180	0.0149
0.00971	0.01961	0.00971	0.000247	0.0196	0.0600
0.01133	0.02113	0.01133	0.000288	0.0211	0.0745
0.01295	0.02264	0.01295	0.000330	0.0226	0.0900

[a]Microgram (AI) per square centimeter of filter paper disc.
[b]Microgram (AI) per fly.

1977). Resistance ratios were determined by the method of Robertson & Preisler (1992).

To determine if a relationship existed between resistance ratios and elevation, a regression analysis was conducted (PROC REG) (SAS Institute 1992). The variable elevation was considered to be a random effect. The diazinon data from 1995 and 1996 were combined for analysis because the susceptible standard 95% confidence limits (CL) at the LC90 estimate overlapped. Due to the very large increases, however, in permethrin resistance ratios from 1995 to 1996, the pyrethroid data from each year were examined separately.

The length of the breeding season at each location was estimated by calculating accumulated degree-days above a threshold. Thomas et al. (1974) and Miller (1977) estimated the developmental threshold at 9.2° and 11.2°C, respectively, based on temperatures recorded at standard weather stations; we chose 10°C as a compromise between the two. Daily maximum and minimum temperatures were obtained from National Oceanic and Atmospheric Administration weather stations at four Wyoming locations: Torrington, Cheyenne, Laramie, and Centennial. Degree-days >10°C were accumulated from 1 January to 30 September at each site by using a modified sine wave routine (Allen 1976). Because current methods for predicting spring emergence and diapause entry are inadequate (Klein and Lancaster 1992), we arbitrarily set 30 September as the end of the breeding season, and used two dates, 1 May and 1 June, as estimates of the beginning of the breeding season. The length of the breeding season at each location was then estimated by subtracting the accumulated degree days at the start of the season from the accumulated degree-days at the end of the season.

Results

All 12 Wyoming horn fly populations were significantly less susceptible to permethrin than was the USDA–ARS Kerrville susceptible strain in 1995; however, no population had as high a resistance ratio as the USDA–ARS Kerrville cyhalothrin-resistant strain (Table 2). The lowest resistance ratio, 3.9, occurred at

Table 2. Insecticide-use history and permethrin dosage used in horn fly filter paper bioassay to determine resistance ratios (RR) for 1995.

Location (elevation, m)	Insecticide history	LD_{50} (95% CL) µg (AI)/cm^2 filter paper	RR
1 Kerrville-susceptible		0.0063 (0.0056–0.0070)	1.0
2 (2,400)	None	0.0105 (0.0056–0.0154)	3.9*
3 (2,100)	None	0.0243 (0.016–0.0325)	15.0*
4 (2,225)	Unknown	0.0237 (0.0158–0.0320)	19.4*
5 (1,740)	None	0.0511 (0.0390–0.0697)	31.5*
6 (1,800)	Pyrethroid & OP ear tags alternating years	0.0344 (0.0253–0.0463)	32.8*
7 (2,225)	Unknown	0.0191 (0.0084–0.0299)	36.0*
8 (1,370)	Methoprene mineral block	0.0753 (0.0580–0.1026)	80.9*
9 (1,220)	Unknown	0.1334 (0.1055–0.1743)	100.3*
10 (1,800) USDA-Main	None	0.1074 (0.0785–0.1782)	112.6*
11 (1,585)	OP backrubber each year	0.0590 (0.0400–0.0837)	160.2*
12 (1,220)	Unknown	0.1548 (0.1259–0.1930)	204.2*
13 (1,800) USDA-East Unit	None	0.1738 (0.1115–0.2755)	516.8*
14 Kerrville cyhalothrin resistant		3.4725 (2.8587–4.0202)	761.1*

*Differs significantly from the USDA—ARS Kerrville-susceptible strain at 95% CL by method of Robertson & Preisler (1992).

location 2, which was at the highest elevation (2,400 m) within 2 km of Albany, Wyoming.

The highest permethrin resistance ratio detected in a Wyoming population, 516.8, was found at location 13, which was the USDA–ARS East Unit near Cheyenne, Wyoming (≈1,800-m elevation). Flies from the lowest elevation (1,220-m elevation), location 12, near Glendo, Wyoming, had the second-highest resistance ratio, 204.2. Flies from location 10, which was the USDA–ARS Main ranch, near Cheyenne had a resistance ratio of 112.6 (1,800-m elevation).

In 1995, six Wyoming horn fly populations were found to be significantly more susceptible to diazinon than was the USDA–ARS Kerrville-susceptible strain (Table 3). However, three populations were found to be significantly more resistant to diazinon than was the USDA–ARS Kerrville-susceptible strain.

As in 1995, all Wyoming horn fly populations tested for permethrin resistance in 1996 were significantly more resistant than the USDA–ARS Kerrville-

Table 3. Insecticide-use history and diazinon dosage used in horn fly filter paper bioassay to determine resistance ratios (RR) for 1995.

Location (elevation, m)	Insecticide history	LD_{50} (95% CL) μg (AI)/cm^2 filter paper	RR
1 Kerrville-susceptible		0.0155 (0.0134–0.0167)	1.00
3 (2,100)	None	0.0069 (0.0062–0.0076)	0.60*
4 (2,225)	Unknown	0.0061 (0.0055–0.0068)	0.61*
5 (1,740)	None	0.0075 (0.0067–0.0084)	0.73*
8 (1,370)	Methoprene mineral block	0.0090 (0.0085–0.0095)	0.73*
2 (2,400)	None	0.0094 (0.0068–0.0119)	0.75*
10 (1,800) USDA-Main	None	0.0083 (0.0077–0.0090)	0.83*
7 (2,225)	Unknown	0.0080 (0.0071–0.0089)	0.87
6 (1,800)	Pyrethroid & OP ear tags alternating years	0.0086 (0.0079–0.0094)	0.92
9 (1,220)	Unknown	0.0104 (0.0092–0.0119)	1.11
12 (1,220)	Unknown	0.0122 (0.0113–0.0132)	1.22*
11 (1,585)	OP backrubber each year	0.0090 (0.0079–0.0101)	1.34*
13 (1,800) USDA-East Unit	None	0.0128 (0.0114–0.0144)	1.37*

*Differs significantly from the USDA—ARS Kerrville-susceptible strain at 95% CL by method of Robertson & Preisler (1992).

susceptible strain (Table 4). In 1996, as in 1995, the Wyoming location with the highest permethrin resistance ratio, 2,056.9, was the USDA–ARS East Unit. The lowest resistance ratio, 22.1, occurred at location 2 at the highest elevation (2,400 m). Unlike the results in 1995, however, horn flies from the USDA–ARS East Unit were found to have a resistance ratio that was larger than that of the USDA–ARS Kerrville cyhalothrin-resistant strain (1,766.7). Based on topical application, the permethrin resistance ratio of the USDA–ARS Kerrville cyhalothrin-resistant strain was 1,745.9 (LD_{50} = 0.25389, 95% CL = 0.25389–0.30280).

Horn flies collected from locations 2 and 11, south of Rock River, Wyoming, which were at the highest elevations, were significantly more susceptible to fenthion than was the USDA–ARS Kerrville susceptible strain (Table 5). Five horn fly populations demonstrated significantly greater resistance ratios to fenthion than did the USDA–ARS Kerrville-susceptible strain. With the exception of population 17, a Nebraska herd at an elevation of 1,065 m, locations with fenthion resistance were at the higher elevations (1,800 to 2,225 m). Horn fly susceptibility to fenthion at all remaining locations was not significantly different from the USDA–ARS Kerrville-susceptible strain.

Table 4. Insecticide-use history and permethrin dosage used in horn fly filter paper bioassay to determine resistance ratios (RR) for 1996.

Location (elevation, m)	Insecticide history	LD_{50} (95% CL) µg (AI)/cm^2 filter paper	RR
1 Kerrville susceptible		0.0015 (0.0004–0.0024)	1.0
2 (2,400)	1% permethrin pour-on 1995	0.0252 (0.0154–0.0376)	22.1*
3 (2,195)	Unknown	0.0718 (0.0503–0.1002)	166.9*
4 (1,570)	Ivomec each year	0.0726 (0.0393–0.1149)	184.0*
5 (1,600)	Co-Ral (96), pour-on pyrethroid	0.0718 (0.0519–0.0972)	225.1*
6 (2,225)	Malathion mosquito trials on ranch	0.0909 (0.0646–0.1272)	231.4*
7 (2,195)	None (4 yr)	0.0462 (0.0284–0.0691)	245.8*
8 (1,980)	Methoprene mineral block	0.2094 (0.1498–0.2957)	451.9*
9 (1,800)	Ectrin ear tags (95, 96)	0.4966 (0.3581–0.6603)	455.8*
10 (1,280)	OP dust bag each year	0.1181 (0.0773–0.1829)	546.8*
11 (2,285)	Dominator (95), Terminator (96)	0.5663 (0.3686–0.9322)	692.2*
12 (1,800) USDA-Main	None	0.0915 (0.0593–0.1395)	795.8*
13 (1,430)	Warbex each fall, Ivomec (bulls)	0.0229 (0.0030–0.0594)	1009.1*
14 (1,800)	Saber Extra ear tags (96), Del Phos	0.3930 (0.2621–0.6528)	1695.8*
15 Kerrville Cyhalothrin-resistant		11.161 (9.9883–11.8974)	1766.7*
16 (1,800) Unit USDA-East	None	0.1700 (0.1086–0.2953)	2056.9*

*Differs significantly from the USDA—ARS Kerrville-susceptible strain at 95% CL by method of Robertson & Preisler (1992).

Three horn fly populations were significantly more susceptible to diazinon than was the USDA–ARS Kerrville-susceptible strain (Table 6). Horn fly populations from five locations demonstrated significantly greater resistance ratios to diazinon than did the USDA–ARS Kerrville-susceptible strain. The horn fly population from Nebraska, elevation 1,065 m, which showed the greatest fenthion resistance ratio, did not differ significantly from the USDA–ARS Kerrville-susceptible strain in diazinon susceptibility.

Based on regression analysis, no significant relationship was detected between vation and resistance ratios to these insecticides. The regression analysis of

Table 5. Insecticide-use history and fenthion dosage used in horn fly filter paper bioassay to determine resistance ratios (RR) for 1996.

Location (elevation, m)	Insecticide history	LD_{50} (95% CL) μg (AI)/cm^2 filter paper	RR
2 (2,400)	1% permethrin pour-on 1995	0.0103 (0.0089–0.0119)	0.30*
11 (2,285)	Dominator (95), Terminator (96)	0.0134 (0.0112–0.0157)	0.59*
13 (1,430)	Warbex each fall, Ivomec (bulls)	0.0133 (0.0104–0.0164)	0.73
9 (1,800)	Ectrin ear tags (95, 96)	0.0072 (0.0023–0.0126)	0.77
3 (2,195)	Unknown	0.0131 (0.0094–0.0171)	0.85
1 Kerrville susceptible		0.0238 (0.0207–0.0272)	1.00
5 (1,600)	Co-Ral (96), pour-on pyrethroid	0.0138 (0.0103–0.0174)	1.09
10 (1,280)	OP dust bag each year	0.0108 (0.0055–0.0168)	1.26
12 (1,800) USDA-Main	None	0.0117 (0.0079–0.0157)	1.37
8 (1,980)	Methoprene mineral block	0.0093 (0.0050–0.0141)	1.40
4 (1,570)	Ivomec each year	0.0155 (0.0111–0.0202)	1.43
7 (2,195)	None (4 yr)	0.0181 (0.0131–0.0235)	1.46*
14 (1,800)	Saber Extra ear tags (96), Del Phos	0.0176 (0.0140–0.0213)	1.46*
16 (1,800) USDA-East Unit	None	0.0143 (0.0101–0.0188)	1.48*
6 (2,225)	Malathion mosquito trials on ranch	0.0238 (0.0179–0.0301)	1.81*
17 (1,[illegible]65) GSL-Nebraska	Unknown	0.0022 (0.0000–0.0100)[a]	2.25*

*Differs significantly from the USDA—ARS Kerrville-susceptible strain at 95% CL by method of Robertson & Preisler (1992).

[a]The 90% CI.

Table 6. Insecticide-use history and diazinon dosage used in horn fly filter paper bioassay to determine resistance ratios (RR) for 1996.

Location (elevation, m)	Insecticide history	LD_{50} (95% CL) µg (AI)/cm^2 filter paper	RR
2 (2,400)	1% permethrin pour-on 1995	0.0083 (0.0074–0.0092)	0.73*
7 (2,195)	None (4 yr)	0.0082 (0.0073–0.0090)	0.75*
12 (1,800) USDA-Main	None	0.0092 (0.0083–0.0101)	0.84*
3 (2,195)	Unknown	0.0088 (0.0078–0.0098)	0.96
8 (1,980)	Methoprene mineral block	0.0092 (0.0074–0.0106)	0.99
1 Kerrville susceptible		0.0181 (0.0171–0.0189)	1.00
9 (1,800)	Ectrin ear tags (95, 96)	0.0067 (0.0052–0.0081)	1.03
17 (1,065) GSL-Nebraska	Unknown	0.0203 (0.0087–0.0118)	1.18
4 (1,570)	Ivomec each year	0.0117 (0.0103–0.0132)	1.27
5 (1,600)	Co-Ral (96), pour-on pyrethroid	0.0135 (0.0122–0.0148)	1.39*
14 (1,800)	Saber Extra ear tags (96), Del Phos	0.0117 (0.0105–0.0128)	1.47*
11 (2,285)	Dominator (95), Terminator (96)	0.0113 (0.0088–0.0139)	1.49*
10 (1,280)	OP dust bag each year	0.0103 (not generated)	1.59*
6 (2,225)	ULV malathion mosquito sprays	0.0085 (0.0062–0.0124)	1.77*

*Differs significantly from the USDA—ARS Kerrville-susceptible strain at 95% CL by method of Robertson & Preisler (1992).

permethrin resistance ratios showed no relationship between elevation and resistance ratio in either 1995 ($P = 0.39$, df = 10) or 1996 ($P = 0.41$, df = 12) (Figs. 1 and 2). The regression analysis of the combined years for diazinon demonstrated no relationship between elevation and resistance ratio ($P = 0.20$, df = 23) (Fig. 3). The regression analysis of fenthion resistance ratios showed no relationship between elevation and resistance ratio in 1996 ($P = 0.09$, df = 13) (Fig. 4).

The accumulated degree-days at four Wyoming elevations are shown in Table 7 for each elevation and year. The accumulated degree days on each date declined as elevation increased. The length of the horn fly breeding season, as estimated by the differences in accumulated degree days between either 1 May or 1 June and September 30 also declined with elevation. Using 1 May or 1 June as the starting date had relatively little influence on the relative length of the fly breeding season at each location. The relative length of the breeding season at Chey-

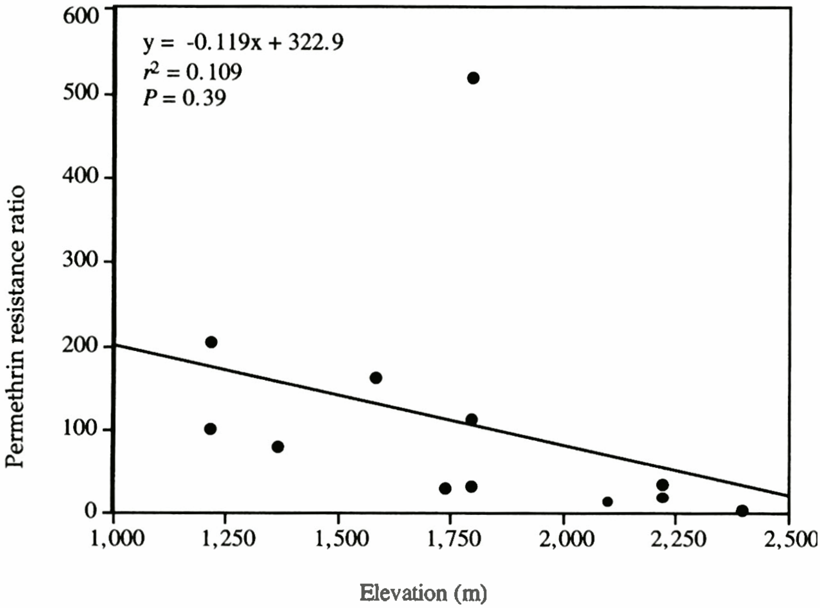

Fig. 1. Relationship between permethrin resistance ratios of horn flies at different elevations (1995).

enne, Laramie, and Centennial in 1995 was 82, 57, and 47% that of Torrington if 1 May was used as the beginning of the fly season, and was 84, 58, and 49% if 1 June was used at the beginning of the fly season. In 1996, the relative length of the horn fly breeding season at Cheyenne, Laramie and Centennial was 77, 59, and 50% that of Torrington, and was 78, 60, and 51% if 1 June was used as the starting date.

Discussion

Methods of testing for adult horn fly resistance have been reported in the literature. In all methods, technical formulations of insecticides were either introduced to the flies on dry surfaces (Sheppard 1984; Schmidt et al. 1985; Cilek & Knapp 1986; Sheppard & Hinkle 1986; Burg et al. 1995) or as a topical application (Harris 1964; Sheppard 1983). In the current study, formulated insecticides were used to determine resistance ratios. This method was chosen because of the similarity to formulations to which wild horn flies are normally exposed.

The resistance ratios determined using this method tended to be higher than those reported by others. Unfortunately, the ratios in our study, cannot be directly compared with those in the literature. Comparisons examining the various assay techniques and the formulated filter paper method by using identical susceptible and field populations of horn flies have not been conducted. Also, many of these published studies are now several years old; horn fly populations have been under selection pressure and their current resistance status is unknown.

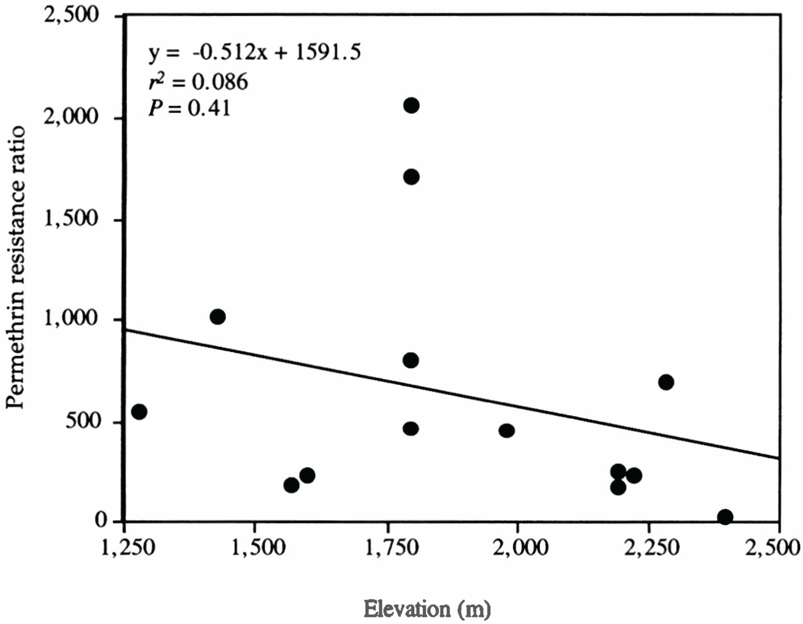

Fig. 2. Relationship between permethrin resistance ratios of horn flies at different elevations (1996).

The cattle at the USDA–ARS East Unit had not been exposed to insecticides for several years prior to this research, however, flies examined from this location had the highest permethrin (516.8) and diazinon (1.37) resistance ratios. A ranch adjacent to the USDA–ARS East Unit did, however, have a history of heavy insecticide use that included both a pyrethroid and an organophosphate. A history of this ranch is documented in Kaufman (1997). The highly resistant horn fly population detected at the USDA–ARS East Unit may have originated on cattle shipped into Wyoming from areas with highly resistant horn fly populations or developed in the native flies on the adjacent herd. It was not possible to assay the horn flies on this herd, thus it is not possible to determine whether this herd was a source of resistant flies to the USDA–ARS East Unit.

Roush & Daly (1990) stated that insect dispersal from heavily treated habitats can increase resistance in lightly treated sites. Thus, it is possible to find relatively high frequencies of resistance at locations where hosts have not been treated.

Sheppard & Joyce (1992) reported large increases in resistance ratios for pyrethroids in areas where lambdacyhalothrin was used from 1986 to 1990 in Georgia. This resistance-ratio increase was accompanied by an increase in ratios in their "no-pyrethroid area," which was 3 km from any known pyrethroid-treated cattle. Horn flies in nearby areas had remained susceptible during 6 yr (1980–1985) of pyrethroid (fenvalerate and permethrin) ear-tag use at the testing location. Following the use of cyhalothrin, however, the tolerance of the horn flies to fenvalerate increased significantly. Sheppard & Joyce (1992) attributed the resistance phenomena to long flights by biologically significant numbers of horn flies.

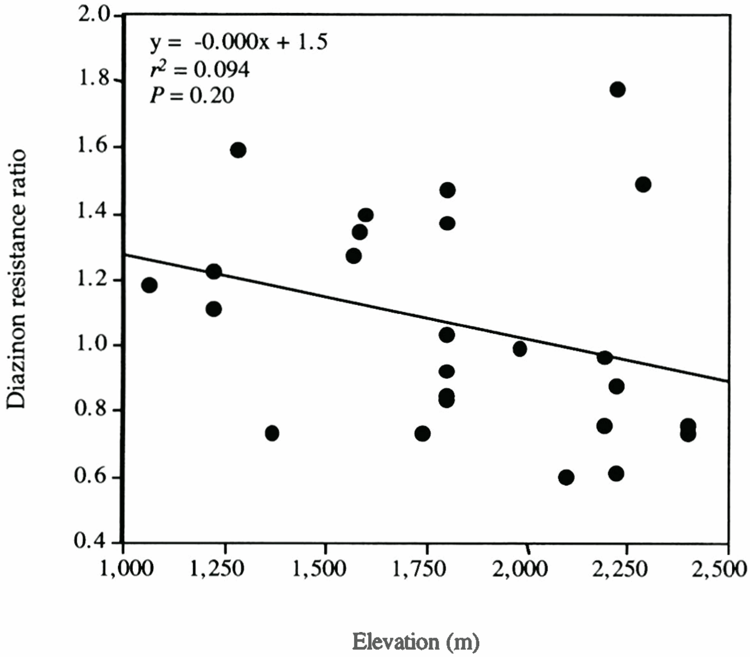

Fig. 3. Relationship between diazinon resistance ratios of horn flies at different elevations (1995 and 1996).

In a mark–release study, Sheppard (1994) reported observing marked horn flies at distances of 5 km shortly after release. Kinzer & Reeves (1974) reported observing marked horn flies at distances as far as 11.8 km. However, as the distance from a source herd increases the likelihood of biologically significant numbers of horn flies locating the same herd of cattle will certainly decrease. In the Sheppard & Joyce (1992) study, fenvalerate resistance developed from flies emigrating from distances >3 km. The application of insecticides on the heifers adjacent to the USDA–ARS East Unit may have maintained a high level of resistance in those flies. Although emigration from additional ranches is possible, it is likely that the adjacent herd is the primary source of migrating flies, as there were no other permanently pastured cattle herds within 8 km of either ranch.

It was noted early in the 1996 horn fly season that permethrin resistance ratios of field-collected horn flies were 4 to 7 times greater than in 1995. Following a repeated examination of flies from the USDA–ARS East Unit on 2, 10, and 19 July 1996 it was noted that the permethrin resistance ratio had increased from 516.8 in 1995 to 2,056.9 in 1996. The susceptible standard base dosages determined for permethrin in 1996 were identical to those from the previous year. Diazinon resistance ratios in field-collected horn fly populations did not show a corresponding increase from 1995 to 1996. Based on reexamination of bioassay procedures and resistance ratio comparisons of populations examined in both years, it would appear that permethrin resistance levels in horn fly populations had increased from the previous year.

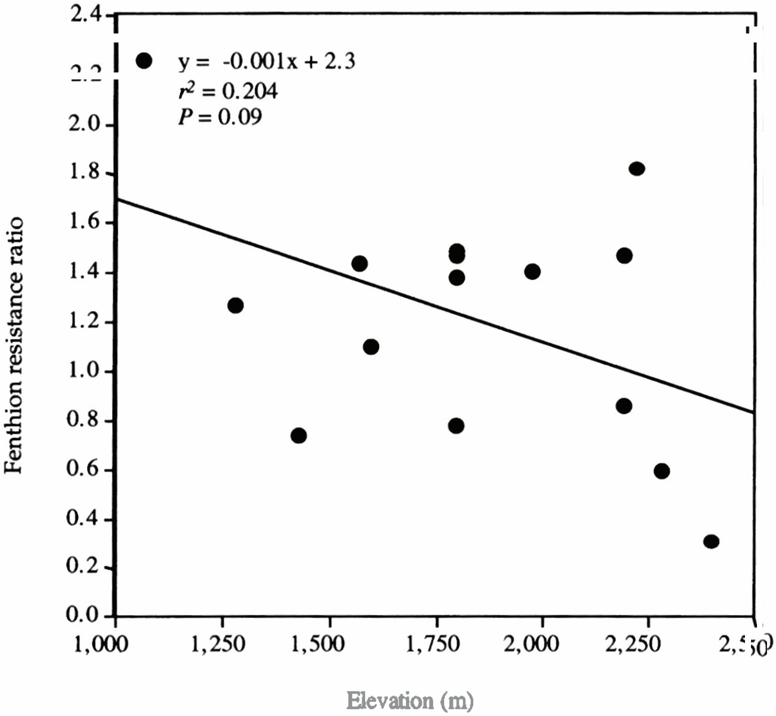

Fig. 4. Relationship between fenthion resistance ratios of horn flies at different elevations (1996).

The large increase in permethrin resistance ratios from 1995 to 1996 in flies from the USDA–ARS Kerrville cyhalothrin-resistant strain was confirmed using a topical application assay in 1996. The permethrin resistance ratio obtained using topical application (1,745.9) was very similar to that obtained using the filter paper assay (1,766.7). The similar results of both assay techniques on the same two colonies of flies support the accuracy of the filter paper technique at high doses with permethrin.

It is theorized that horn fly insecticide resistance development in Wyoming has been delayed by naturally low fly populations, resulting from a reduced number of fly generations produced during the summer (especially at higher elevations). Reduced insecticide treatment of horn flies due to inaccessibility of cattle grazed on the national and state forests also may delay development of resistance.

Investigations of horn fly densities at 800-, 1,800-, and 2,400-m elevations revealed densities to be lowest at the 2,400-m elevation (Kaufman 1997). Further, the horn fly developmental model results give a crude indication of the relative length of the fly breeding season at each location, assuming the same dates for spring emergence and diapause entry. In all probability, spring emergence occurs later and diapause induction earlier at higher elevations compared with lower elevations, so our results probably underestimate the magnitude of the differences in the length of the breeding season between elevations. For every one horn

Table 7. Accumulated degree-days >10°C on selected dates at four locations.

Year	Date	Elevation (m)[a] 1,200	1,800	2,100	2,400
1995	1 May	122	73	32	18
	1 June	190	106	55	36
	1 July	428	275	169	139
	1 August	787	582	377	314
	1 September	1190	955	644	528
	30 September	1397	1120	760	624
	Rel. Time May 1[b]	1.00	0.82	0.57	0.47
	Rel. Time June 1[b]	1.00	0.84	0.58	0.49
1996	1 May	125	61	29	16
	1 June	265	159	103	76
	1 July	596	395	281	227
	1 August	960	709	538	442
	1 September	1316	991	747	631
	30 September	1511	1135	849	712
	Rel. Time May 1[b]	1.00	0.77	0.59	0.50
	Rel. Time June 1[b]	1.00	0.78	0.60	0.51

[a]Degree-days calculated from daily maximum and minimum temperatures

fly generation completed at the 1,200 m elevation in 1995, only 0.47 generations were completed at the 2,400 m elevation. This finding would indicate that horn fly resistance selection can occur over twice as fast at the lower elevation.

Cattle grazed on forest allotments are likely to be ear tagged early in the season, often at branding time, long before horn fly populations begin to increase. Because of this practice, much of the insecticide in the ear tag is released and lost before adult horn flies have emerged from diapause. The inaccessibility of cattle for treatment combined with reduced fly densities may discourage insecticide use at the high-grazing elevations throughout the state.

The use of cattle ear tags has been strongly implicated in the development of horn fly resistance to several pyrethroids and to the organophosphate stirofos (Quisenberry et al. 1984; Sheppard 1984; Byford et al. 1985; Sparks et al. 1985). Considering the small percentage of cattle treated with ear tags in Wyoming (Legg et al. 1992), similarly low horn-fly-resistance levels would be expected. The permethrin resistance ratios for horn fly populations in Wyoming do not support this conclusion, but the low resistance ratios for fenthion and diazinon do correspond with the low organophosphate use on Wyoming cattle.

A relationship between elevation and resistance ratios was not detected in our study with any of the insecticides examined suggesting that factors other than the altitude at which cattle are grazed have a stronger influence on horn fly resistance development. Use of insecticides by ranchers without consideration of economic injury levels or production costs may have contributed to the lack of a statistical relationship. The hypothesis of lower resistance levels at higher elevations is based on the presumption that ranchers at higher elevations with lower horn fly densities would be less likely to treat their cattle. Apparently, this is not the case, as evidenced by the insecticide use histories. Movement of cattle and their flies from lower to higher elevations in the summer also may result in increasing the resistance ratios at higher elevations as well as horn fly dispersal within and between various elevations. The movement of susceptible flies from higher to lower elevations is unlikely to have a reciprocal effect because cattle usually are not moved from the higher elevations until early October, at which time flies have either died or entered diapause.

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