A STUDY OF THE EFFECT OF SOME OKLAHOMA ENVIRONMENTAL FACTORS AND THE LARVICIDAL ABILITY OF DIMETHYL PHOSPHATE OF α-METHYLBENZYL 3-HYDROXY-CIS-CROTONATE 2,2-DICHLOROVINYL PHOSPHATE (VIP-DICHLORVOS) ON THIRD STAGE *ANCYLOSTOMA CANINUM* (ERCOLANI, 1859) LARVAE

by

LAWRENCE DAVID SHAMIS

Bachelor of Science in Agriculture

University of Arizona

Tucson, Arizona

1974

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
July, 1977

A STUDY OF THE EFFECT OF SOME OKLAHOMA ENVIRONMENTAL FACTORS OF THE LARVICIDAL ABILITY OF DIMETHYL PHOSPHATE OF α-METHYLBENZYL 3-BYDROXY-CIS-CROTONATE 2,2-DICHLOROVINYL PHOSPHATE (VIP-DICHLORVOS) ON THIRD STAGE *ANCYLOSTOMA CANINUM* (ERCOLANI, 1859) LARVAE

Thesis Approved:

Helen E. Jordan
Thesis Advisor

John T. Homer

R. Allan Tacker

Norman N. Durham
Dean of the Graduate College

ACKNOWLEDGMENTS

Research funds and equipment were supplied by the Department of Veterinary Parasitology, Microbiology and Public Health and a research grant from Pet Chemicals, Inc., Miami Springs, Florida.

The author would like to express his gratitude to the members of his committee who guided him in this research project. He would like to express special thanks to Dr. Helen E. Jordan, his major advisor, for her guidance in conducting the research and preparation of this manuscript. The author wishes to express his gratitude to Dr. John T. Homer and Dr. A. Alan Kocan for counseling during the research and for their aid in completion of this manuscript.

The author would also like to thank Dr. Ralph G. Buckner, Department of Medicine and Surgery for his guidance on clinical aspects of this project, and Dr. R. D. Morrison, Department of Statistics who aided in the analysis of the data.

My thanks are also due to the rest of the faculty, secretaries, and graduate students in this department for their cooperation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

Prior to the turn of the century, the dog hookworm *Ancylostoma caninum* has long been recognized for its widespread geographic prevalence and pathogenicity. Acute parasitism of the host can cause marked anemia, hemorrhagic gastro-enteritis and many times death.

A. caninum has a direct life cycle with the egg, hatching in the external environment. The infective third stage larvae can gain entry into its host from the external environment either by percutaneous or per os route.

Since the egg can develop in two days under optimum conditions and can survive for a variable length of time, a contaminated pen or run can be a source of continuous exposure of the parasite for the host. Thus treating the host with an effective anthelmintic may result in only a short period of being free of the parasite due to a contaminated environment which allows for continual exposure to the hookworm and a constant health problem for the host (1). Therefore, research on immunity, environmental influences, and the effects of various chemicals (as larvicides) on free-living larval stages is very important to providing information for better control programs.

The purpose of this investigation was to determine the environmental effects of Oklahoma climate on free-living stages of *Ancylostoma*

caninum using the parameters of larval development time and their subsequent survival in natural environmental conditions.

In addition, the larvicidal value of spraying the environment with Dimethyl phosphate of α-methylbenzyl 3-hydrox-cis-cromate 2,2-dichlorovinyl phosphate (VIP-Dichlorvos) on third stage *Ancylostoma caninum* was determined.

CHAPTER II

LITERATURE REVIEW

The optimum temperature for Ancylostoma caninum larval development has been established as ranging from 25-35°C (2)(3). Levine reported the optimum temperature for larval development was higher than for larval survival. Optimum embryonation occurred at 36.8 ± 2.8^{o}C in less than 24 hours (4). Several workers have experimentally concluded that high temperatures increase the rate of development and metabolic activity of strongyle larvae which consequently increases stored food utilization and thereby shortening larval life (4)(5)(6).

Other factors are known to affect the free-living stages of the hookworm. As early as 1917, Nicoll found that sunlight of sufficient intensity will kill human hookworm eggs and larvae (7). Consistently lower larval counts have also been reported by Mark (8) for A. caninum due to the effect of direct sunlight. McCoy found that hatching of larvae occurred in a pH range of 4.0-10.8. Larvae hatched above pH 10 died almost instantly (3). Using varying concentrations of Polyethylene glycol in soil, Parking showed various degrees of osmotic pressure to greatly affect (by dessication) the rate of hatching and survival of Nematodirus battus. It is therefore possible that strongyle eggs and larvae may be susceptible to stress caused osmotic pressure in a natural environment (11).

Survival of other hookworm larvae for a period of 18 months have been noted in the laboratory were maintained at 15.6°C (4). Two strongyles, Ostertagia ostertagi and Cooperia oncophora, were recovered from pasture plots 10-20 months after contamination (12). Mark found that bermuda grass was more suitable for A. caninum larval survival (average 25.6 days) than bare ground, pea gravel, and concrete. Concrete was the least suitable substrate (8)(13).

Many chemicals have been studied for their larvicidal potential against A. caninum. Saturated sodium chloride solution has been found to be larvicidal at the rate of .107 gallons per square foot (14). Sodium hydroxide has been shown effective when used to bring the soil to pH 12 or above (15).

Levine studied 70 different chemicals in the laboratory using incubated horse feces containing strongyle eggs. Some of the most effective were mercuric chloride, nicotine sulphate, and potassium iodide. No information was given as to the effectiveness of chemicals under natural environmental conditions. Among the least effective compounds were DDT, sulfur, and talcum powder (16). Applications of 5 or 10% solutions of acetic acid to cat fecal soil cultures killed over 98% of the canine hookworm larvae in those cultures. No data was given as to its efficiency under natural conditions (17). Hoerlein found sodium borate to be effective when applied to contaminated soil but it was highly toxic to vegetation. Calcium cyanomide was fairly effective but can produce hemolytic jaundice and death in the dog (18). Some organophosphates (Malthion and Co-Ral)* have some larvicidal

---

*Chemagro, Division of Baychem Corp., P.O. Box 4913, Kansas City, MO 64120.

activity in vitro. Tests were conducted on larvae immersed in several different concentrations of the chemical. No studies of these compounds were conducted on a naturally contaminated environment (19).

CHAPTER III

MATERIALS AND METHODS

Outdoor Experimental Facilities

A fenced outdoor bermuda pasture that was approximately one acre and had not been used to hold animals within the past two years or more was used for all the outdoor experiments.

Source of Larvae

Hookworm egg laden feces was obtained from dogs maintained for student surgery at Oklahoma State University, College of Veterinary Medicine, Stillwater, Oklahoma. Only feces with an egg concentration of 2,500 per gram or more was used.

Third Stage Larval Preparation

Infective third stage larvae were cultured in the laboratory by maxing two parts charcoal to one part feces in covered 25 cm diameter flat bottomed watch glasses (22). Cultures were incubated at 27°C for eight days. The larvae were recovered by placing three to four layers of water soaked cheesecloth on the surface of the charcoal media.

The cheesecloth was removed after two hours, as shown to be sufficient time for larval migration by Beaver (1953). The cheesecloth was then Baermannized and the larvae were collected (10).

Recovery of Larvae from Plots and Cultures

Larvae were recovered by placing fecal cultures or plot material on a Kimwipe*-lined screen in a Baermann apparatus (30-40 cm funnel that was previously filled with 35°C water to the wire screen). Then additional water was gently added to the side of the funnel until the sample was covered. After four to six hours, the water from each Baermann apparatus was drawn into 50 ml centrifuge tubes. The suspension was then centrifuged at 1500 rpm for five minutes.

Weather Data

Weather data consisting of daily high and low temperatures and rainfall were obtained from the Oklahoma State University Agronomy Department weather station located 100 meters due south of the experimental area.

Ancylostoma caninum Determination from Larval Samples

The total number of larvae was estimated by taking five 0.1 ml aliquots from a constant volume of 10 ml and counting the larvae in each sample. The samples were then subjected to 3% HCl solution to determine the number of parasitic larvae (20). To obtain the proper acid concentration, 0.1 ml of 6% HCl was added to 0.1 ml of larval suspension. Larvae surviving after 45 seconds of exposure to the acid solution were considered parasitic since free-living nematodes are

---

*Kimberly Clark, Neenah, Wisconsin 54956.

killed within this interval (20). Therefore, 45 seconds after adding HCl to the larvae, the petri dishes were examined under the stereoscopic microscope and motile larvae were counted.

Statistical Analysis

Statistical analysis was conducted by computer using the Statistical Analysis System designed by A. J. Barr and J. H. Goodnight, Department of Statisitics, North Carolina State University, Raleigh, North Carolina, 1972.

Experimental Design

Experiment 1: Egg Contamination Plots

A 4 x 8 foot area was divided into 32 one-foot diameter circle plots. Each plot was circumscribed by a corrugated 15 cm garden edging that was embedded 1.3 cm in the ground. In October 1975, sixteen plots selected randomly were contaminated with feces containing approximately 1.0×10^6 *A. caninum* eggs.

These 16 contaminated plots were divided randomly into four subgroups for treatment. VIP was applied to various developmental stages of eggs and larvae: Group I, larvated eggs; Group II, first stage larvae; Groups III and IV, 10 day and 21 day post-hatch, respectively. Two control plots were left untreated for each of the preceeding subgroups. See Figure 1 for layout of design.

Upon contamination, the plots were checked on a daily basis for larval development by simple flotation procedure using saturated sodium nitrate as a levitation solution. The rate of development was estimated on the basis of range in days each life stage was noted.

1	2 Overwinter Control	3 VIP 10 Days Post Hatch	4 Control 10 Days Post Hatch
5 Overwinter Control	6	7 VIP Larvae (1st & 2nd Stage)	8
9 Control Larvae (1st & 2nd Stage)	10 Overwinter Control	11	12
13 VIP Larvated Eggs	14	15	16
17	18	19 VIP 21 Days Post Hatch	20
21 Overwinter Control	22	23 VIP 21 Days Post Hatch	24 VIP Larvated Eggs
25	26 VIP Larvae (1st & 2nd stage)	27 VIP 21 Days Post Hatch	28 Control Larvated Eggs
29	30 Control 21 Days Post Hatch	31	32

Northern Slope

Southern Slope

Figure 1. Design of 16 Plots Contaminated with 1.0×10^6 *Ancylostoma caninum* Eggs Per Plot in October.

Infection of Dogs with *Ancylostoma caninum* Larval from Experimental Plots of Experiment 1

Ten adult beagle dogs ranging from ages from two (2) to seven (7) years were obtained from the Oklahoma State University hemophiliac colony. None of these dogs had ever shown positive fecal examination for *A. caninum*. Therefore, it was assumed that all ten dogs were hookworm naivé. Prior to infection, corticosteroid azium (dexamethasone) was administered to nine (9) dogs and the tenth served as a control. The dosage was 5 mg/kg body weight I.V. twice daily until differential blood counts showed lymphocytes to be below 500 per cubic milimeter (25).

Each dog was then given the total larvae recovered from one experimental plot, orally. The suspension of larvae was in .75% saline.

All dogs were checked by fecal flotation every other day for 35 days post infection.

Experiment 2: Egg Contaminated Plots - Effect of Topography in Pasture

Outdoor bermuda grass plots made from 15 cm corrugated metal garden edging forming 30 cm diameter circles were placed on ground having northern and southern slopes of 30°. Four plots faced south, and four faced north. These plots were different from the area used in Experiment 1.

Each plot was contaminated with 1.0×10^{6} eggs in feces. Two plots (one north and one south) were monitored daily by simple fecal flotation (see Figure 2).

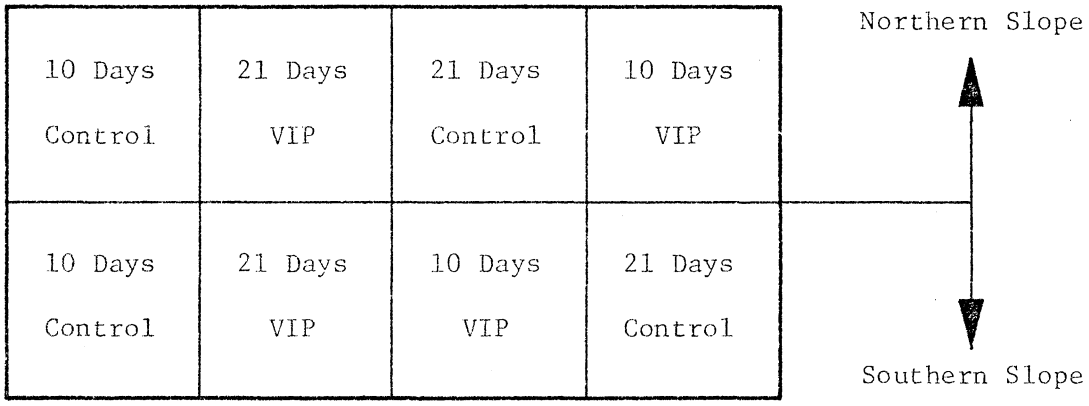


Figure 2. Experimental Design of Eight Plots Contaminated with 1.0×10^6 *Ancylostoma caninum* Eggs Per Plot.

On day 10, one plot on the south and one on the north were treated with VIP according to the manufacturer's directions. After 24 hours the two treated plots and two controls (one from each slope) were recovered and Baermannized.

On day 21, one plot randomly chosen on the south and one on the north slope were treated. Twenty-four hours after treating the two plots, grass and top 1 cm were collected from them and the two remaining controls.

Experiment 3: Third Stage Larval Contamination

Plots

Preparation of Plots. Individual plastic pans approximately 20 cm wide, 30 cm long, and 15 cm deep with holes in the bottom for drainage were used in both the indoor and outdoor trials. For bare ground studies, the bottom of the container was layered first with 1-2 cm of pea sized gravel and then with 5-7 cm of heat sterilized native soil. For grass studies, the containers were prepared as for soil studies with the addition of a layer of bermuda grass on top of the soil. Plugs of grass were taken from a source to be known to be free of all types of mammalian parasitic larvae. The 3-5 mm tall grass and root plugs were cut in the exact measurements of the container. An individual plot is illustrated in Figure 3. The soil and grass was then moistened with water. The plots were allowed to stand overnight before contaminating with larvae.

Duplicate tests were conducted for the larval contamination experiment according to the following procedure:

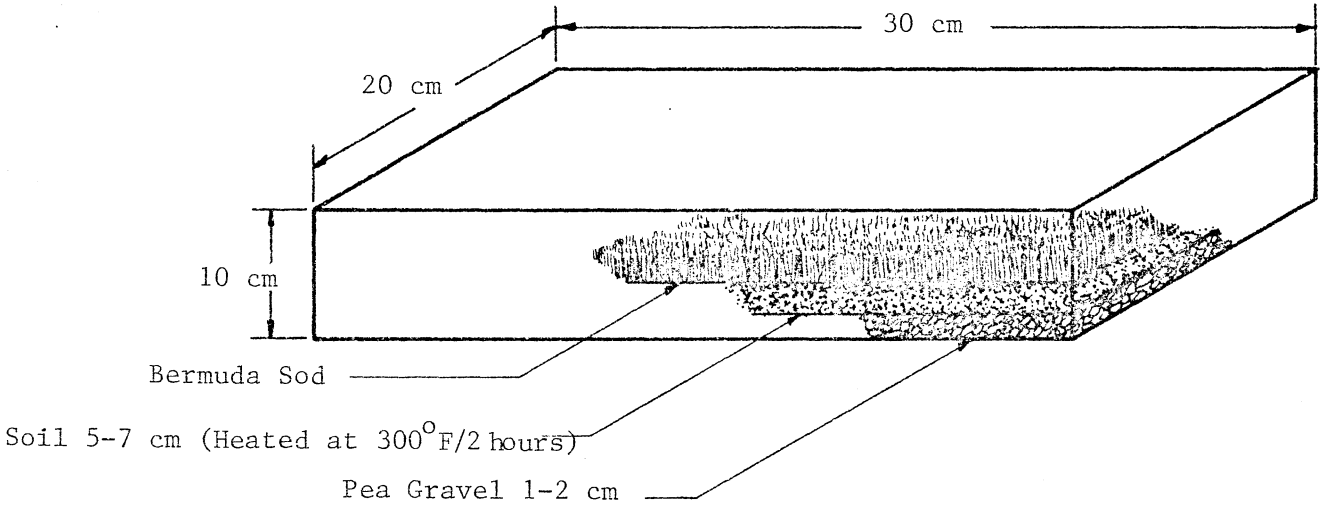


Figure 3. Plot Design for *Ancylostoma caninum* Larval Contamination

In each test, two groups of four grass plots and four bare ground plots in plastic containers that were prepared according to the above procedure were contaminated each with approximately 15,000 third stage A. caninum larval by pouring the suspension in the middle of each plot (21). Immediately after the plots were contaminated one group was placed outdoors and buried within 5 cm of the top rim. The other group of plots was placed in a controlled environmental chamber maintained at 23°C and 70% humidity. Contaminated plots were allowed to sit four hours before treating with VIP. A 1.5% solution in water of the concentrate was applied to two grass and two bare ground plots in both environments at the rate of 180 ml per plot using a plastic house plant sprayer.

In each test, outdoor and environmental chamber trials were run simultaneously. The plots were treated in the afternoon and left undisturbed for 30 hours in a fenced area that would prevent any animals from entering the test area. The environmental condition of the day was recorded (overcast, rainy, bright sun, etc.) and temperature range.

Upon recovery the grass plugs were lifted from the plots and the upper surface placed down on the Kimwipe-lined screen in a Baermann apparatus and larvae collected.

Parasitic larvae were counted using the previously described method. When the bare ground plots were recovered, approximately 2.3 cm deep of soil from the entire plot was removed. The soil was placed on six layers of Kimwipes lining the screen suspended in the Baermann apparatus. The larvae were recovered and the numbers of parasitic larvae determined.

CHAPTER IV

RESULTS

Experiment 1

Larvated eggs were noted from day six to day 13 after contamination. Some hatched larvae were observed on day 10 and until the experiment ended on day 20.

The *A. caninum* egg contamination experimental results are noted in Table 1 and Figure 4. Statistical analysis of this data failed to yield any significant difference in plots regarding the treatment and larval survival by analysis of variance (F_{cal} 0.115; $P > .05$). A trend, however, was noted wherein plots with a northern slope generally produced more infective larvae than those with a southern slope (Figure 2).

The four contaminated overwintering plots failed to yield any infective larvae when checked in the spring.

Figure 5 gives the climatograph for weather data during the period of Experiment 1.

Experimental Infection of Dogs

No dog was ever positive for hookworm infection by fecal flotation. As noted in Table II, no hookworms, adult or larvae, were recovered at necropsy.

TABLE I

THE NUMBER OF *ANCYLOSTOMA CANINUM* LARVAE RECOVERED FROM PLOTS CONTAMINATED WITH 1.0×10^6 EGGS IN EGG CONTAMINATION EXPERIMENT 1

Plot No.	Treatment	Day[1]	No. Larvae Recovered	% Survival[2]
13 (larvated egg)	VIP	13	0	0
24 (larvated egg)	VIP	13	0	0
28 (larvated egg)	Control	13	0	0
7 (hatched larvae)	VIP	17	6400	0.0064
26 (hatched larvae)	VIP	17	90	0.00009
9 (hatched larvae)	Control	17	5400	0.0054
3 (10 days post hatch)	VIP	27	260	0.0026
27 (10 days post hatch)	VIP	27	170	0.00017
4 (10 days post hatch)	Control	27	240	0.0024
19 (21 days post hatch)	VIP	37	0	0
23 (21 days post hatch)	VIP	37	110	0.00110
30 (21 days post hatch)	Control	37	0	0
2 (overwinter)	Control	192	0	0
5 (overwinter)	Control	192	0	0
10 (overwinter)	Control	192	0	0
21 (overwinter)	Control	192	0	0

[1]The number of days post-contamination.
[2]The percent of eggs recovered as larvae.

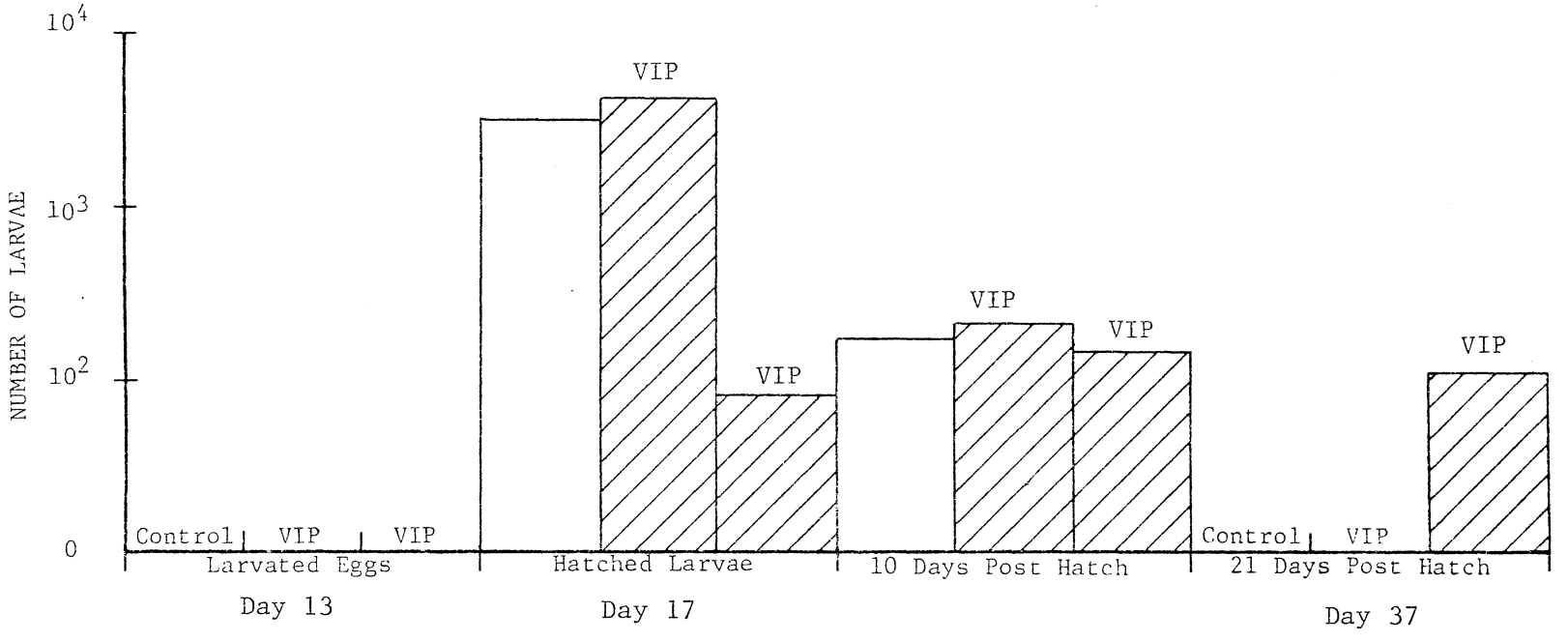


Figure 4. Ancylostoma caninum Infected Larval Recovery for Egg Contamination Plots VIP Treated During Larvated Egg, First and/or Second Stage Larvae, 10, and 21 Days After Hatching.

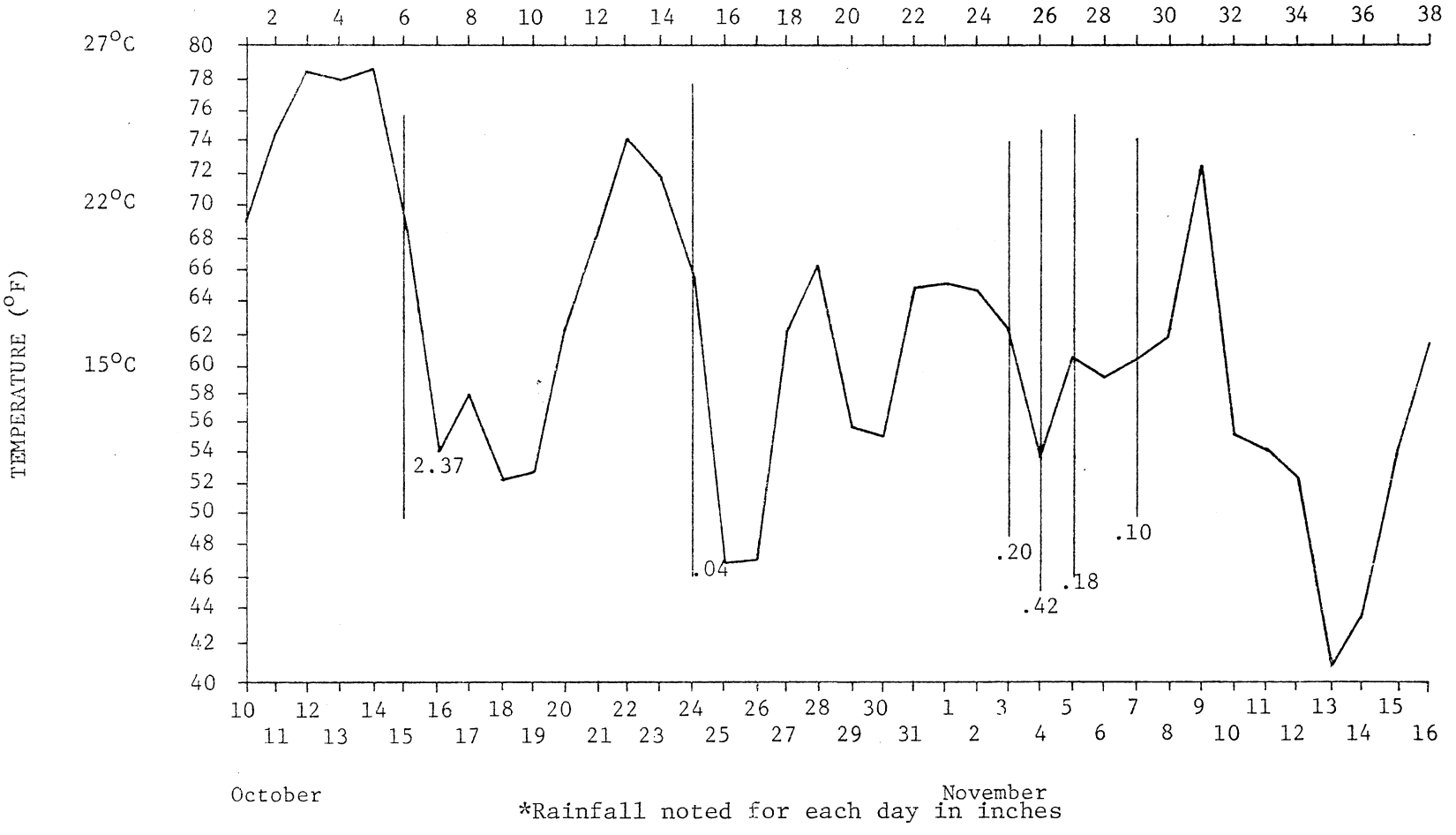


Figure 5. Climatograph for October Larvae Average Ambient Temperature and Rainfall*

TABLE II

TREATMENT, LARVAL DOSE AND THE NUMBER OF WORMS RECOVERED AT NECROPSY FROM DOGS INFECTED WITH THIRD STAGE *ANCYLOSTOMA CANINUM* LARVAE RECOVERED FROM PLOTS IN EXPERIMENT 1

Dog No.	Sex	Treatment	Larval Dose	No. Worms at Necropsy
Y-3	F	Dexamethasone	90	0
U-1	F	Dexamethasone	5400	0
X-3	F	Dexamethasone	260	0
PE-3	F	Dexamethasone	Saline Control	0
E-29	F	Dexamethasone	6400	0
H-10	F	Dexamethasone	260	0
D-5	F	Dexamethasone	240	0
MI-4	F	Dexamethasone	170	0
S-5	M	Sterile Saline Control	110	0
Z-5	M	Dexamethasone	30(free-living)	0

Experiment 2

Larvated eggs were noted from day six to day 12 after contamination. Some hatched larvae were observed from on day 13 and until day 16 at which time the fecal material on the plots were completely dispersed due to environmental effects. This prevented any further monitoring of larval development.

The results of plot treatment and larval recovery are given in Figure 6. Statistical analysis of this data did not yield any significant difference in plots relative to treatment or slope direction by Analysis of Variance (F_{cal} 0.095; $P > .05$).

See Figure 7 for weather data pertaining to this experimental period.

Experiment 3

The results of the first and second test of the infective larval study are given in Table III and Figures 8, 9, 10, and 11. This data was subjected to statistical analysis (Analysis of Variance). The results of the two trials using infective larvae showed VIP larvicide to effectively decrease survival. A significant difference (F_{cal} 37.14; $P < .05$) between treated and control plots were found.

The weather conditions for Trial 1 were dry and the temperature range was 73°-94°. In Trial 2, rainfall of .11 and a temperature range of 74°-100° were recorded. In Trial 2, there was a significant (F_{cal} 19.4; $P < .05$) overall increase in the number of larvae recovered.

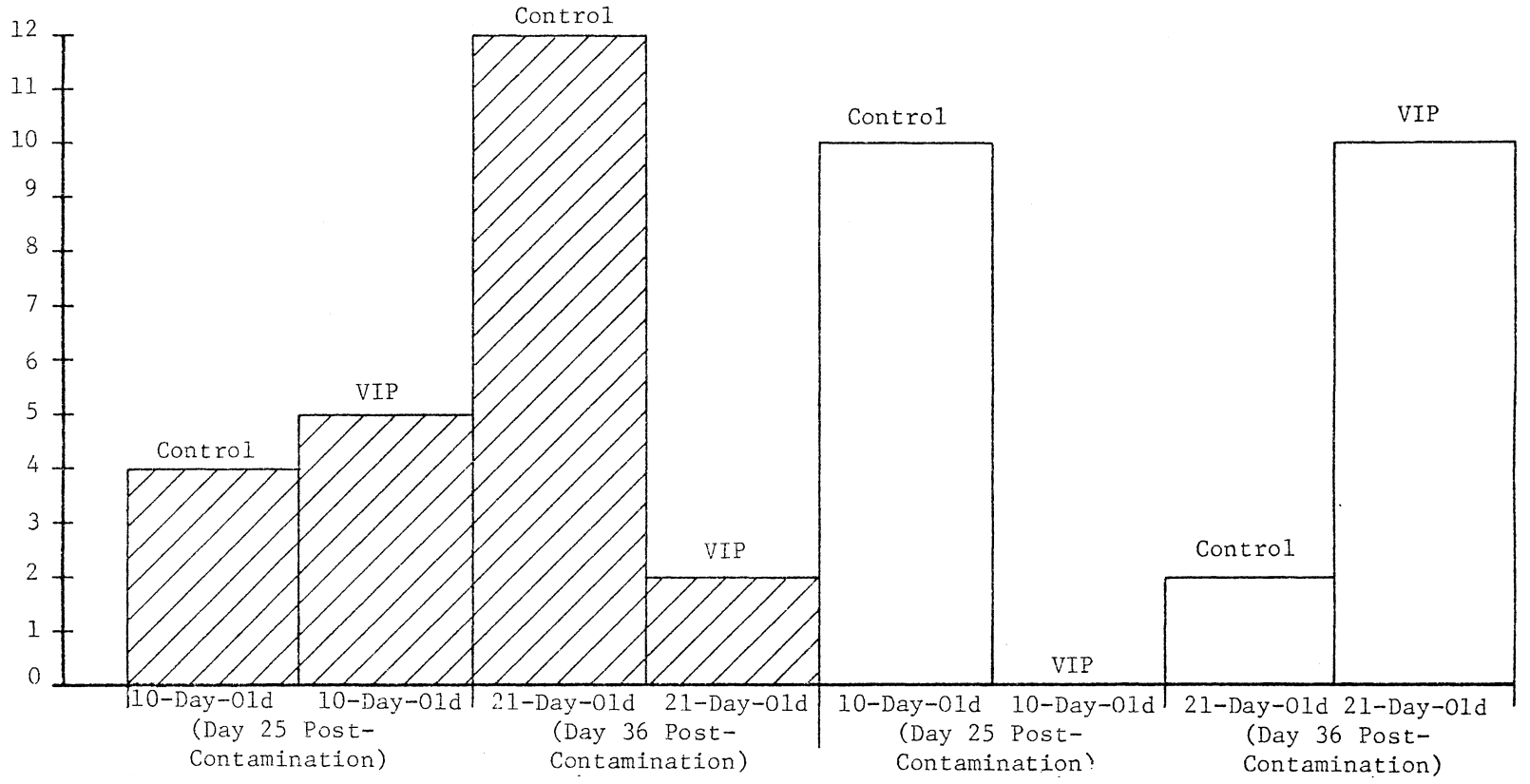
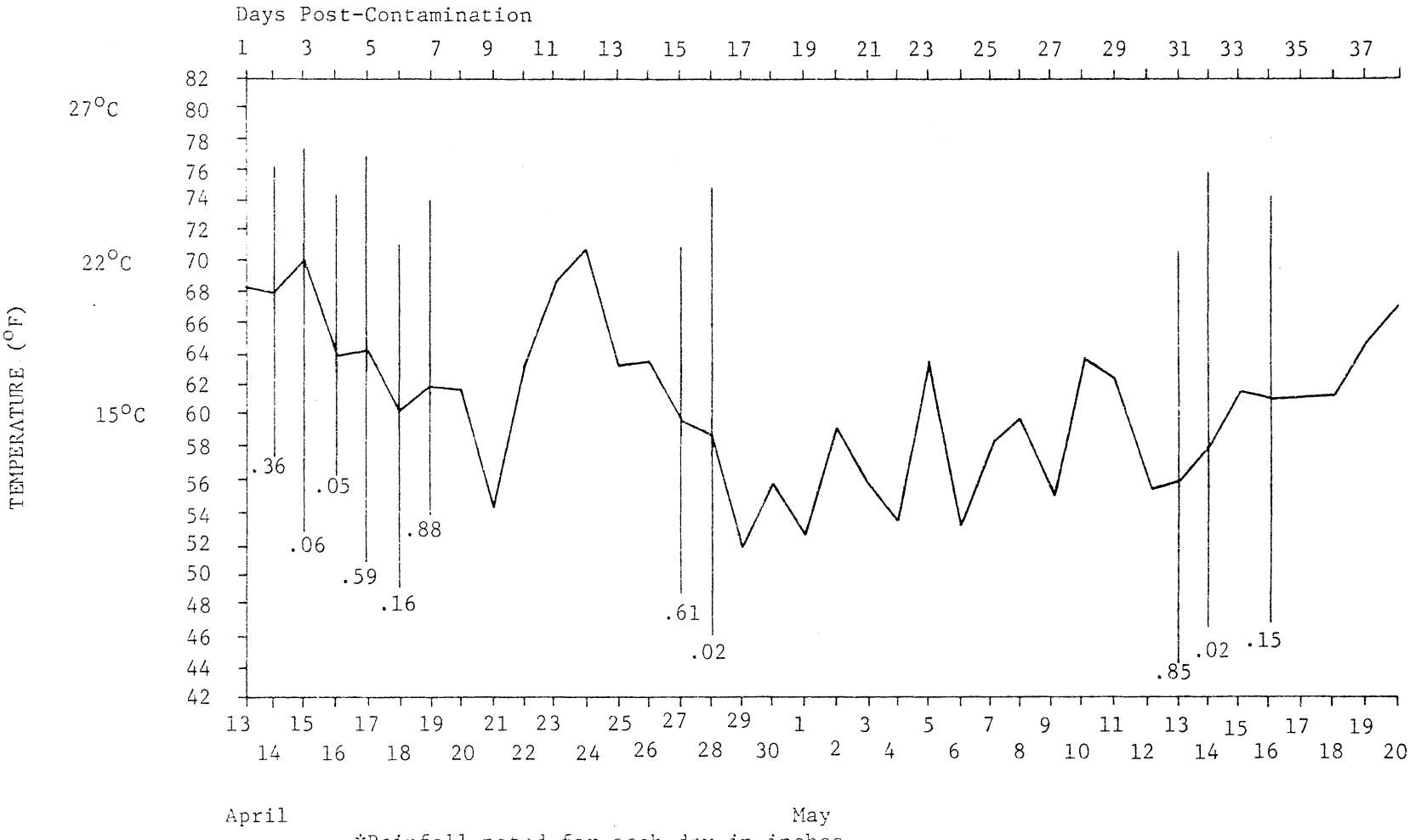


Figure 6. Results of *Ancylostoma caninum* Larval Recovery for Egg Contamination on Northern and Southern Sloping Grass Plots VIP Treated at 10 Days and 21 Days After Hatching



*Rainfall noted for each day in inches.

Figure 7. Climatograph for April and May, 1976, Average Ambient Temperature and Rainfall*

TABLE III

THE NUMBER OF ANCYLOSTOMA CANINUM LARVAE RECOVERED FROM PLOTS IN TRIALS 1 AND 2 OF INFECTIVE LARVAL CONTAMINATION EXPERIMENT

Indoor								Outdoor							
Trial 1															
G-T 97	G-T 79	G-C 556	G-C 500	BS-T 29	BS-T 47	BS-C 253	BS-C 518	G-T 124	G-T 59	G-C 283	G-C 456	BS-T 8	BS-T 3	BS-C 0	BS-C 6
Trial 2															
1199	237	2912	2537	311	578	2853	1190	481	663	656	1558	318	349	2213	1789

Key: G-T = Grass VIP
G-C = Grass Control
BS-T = Bare Soil VIP
BS-C = Bare Soil Control

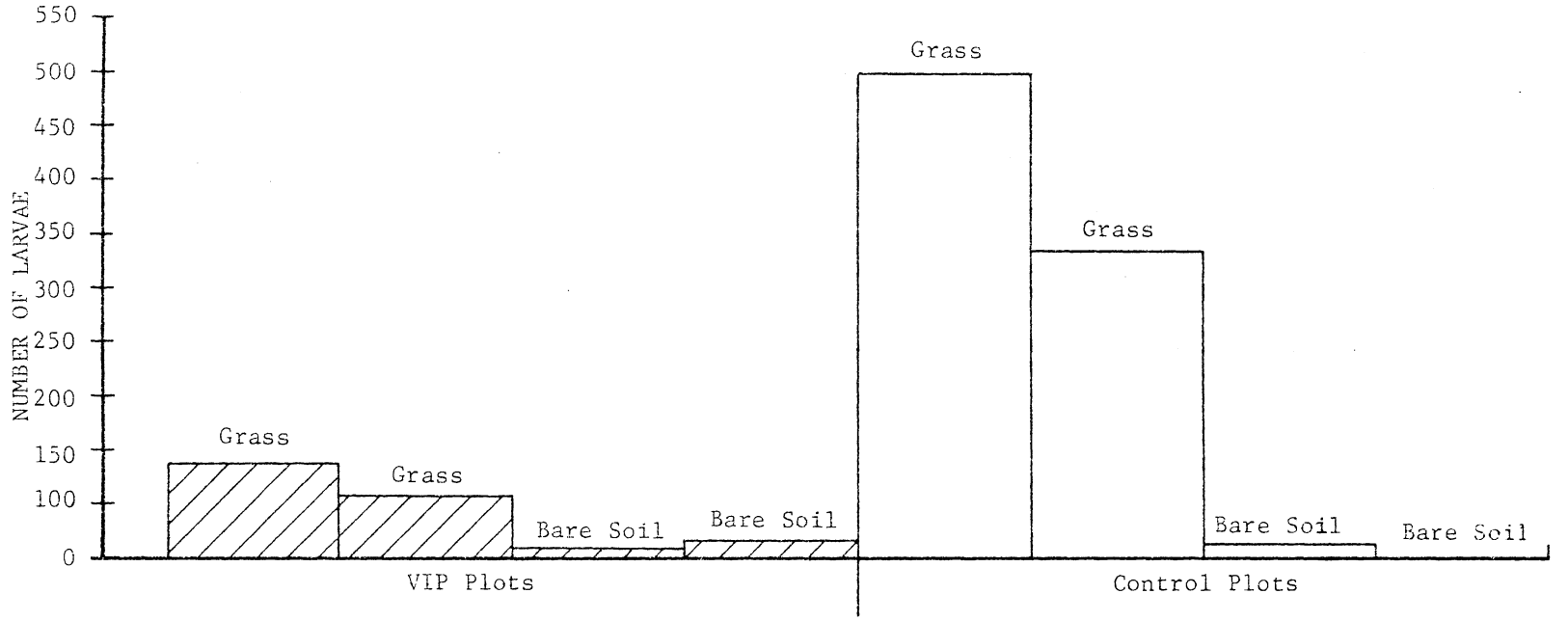


Figure 8. The Number of Ancylostoma caninum Larvae Recovered from Outdoor Grass and Bare Soil Plots Contaminated with 1.5×10^4 Infective Larvae 30 Hours After Treatment (Trial 1)

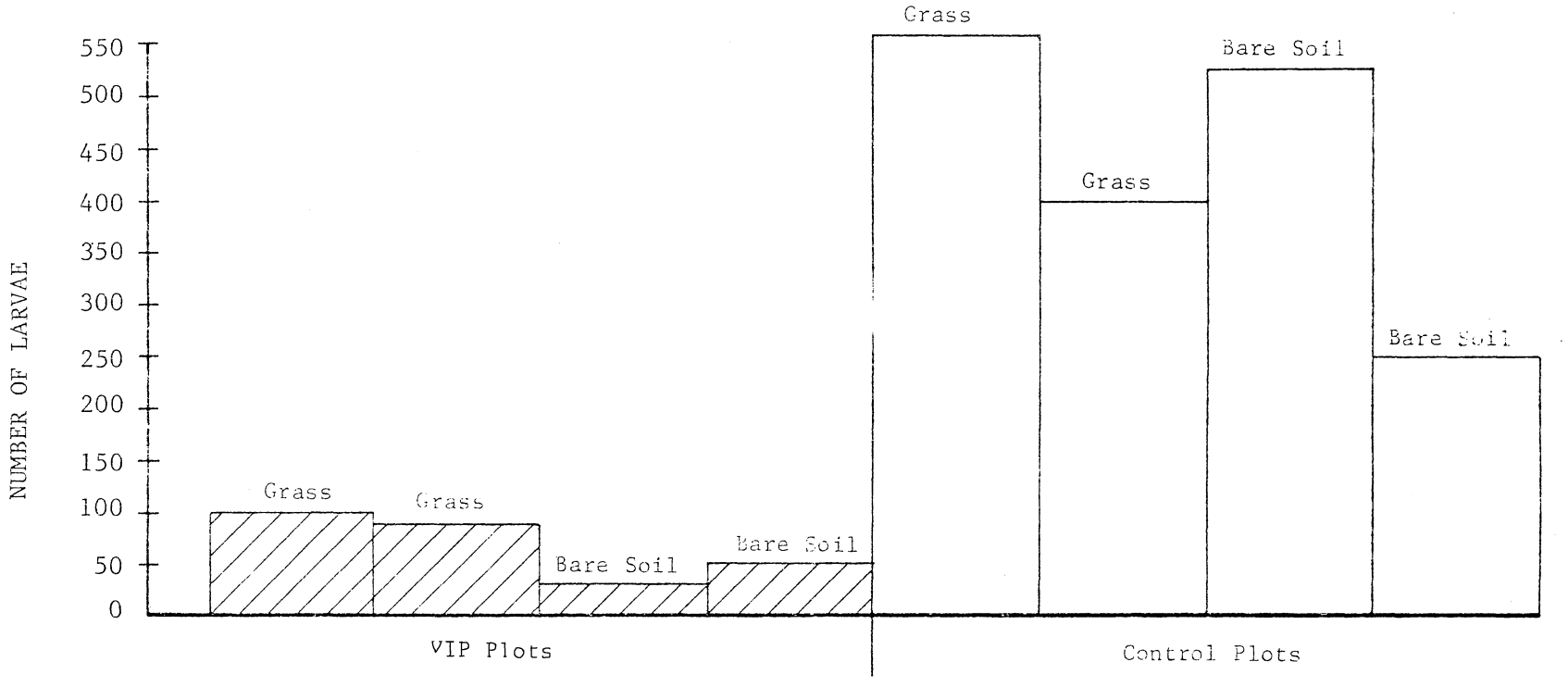


Figure 9. The Number of Ancylostoma caninum Larvae Recovered from Indoor Grass and Bare Soil Plots Contaminated with 1.5×10^4 Infective Larvae 30 Hours After Treatment (Trial 1)

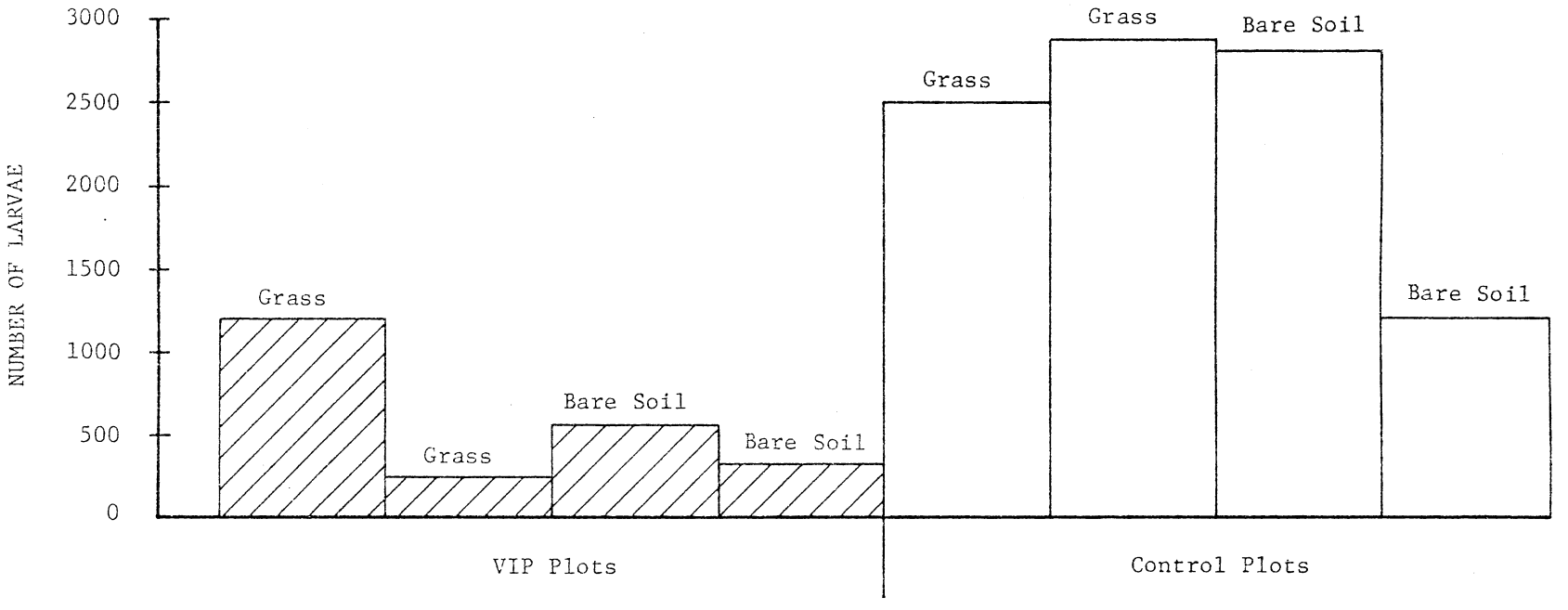


Figure 10. The Number of Ancylostoma caninum Larvae Recovered from Indoor Grass and Bare Soil Plots Contaminated with 1.5×10^4 Infective Larvae 30 Hours After Treatment (Trial 2)

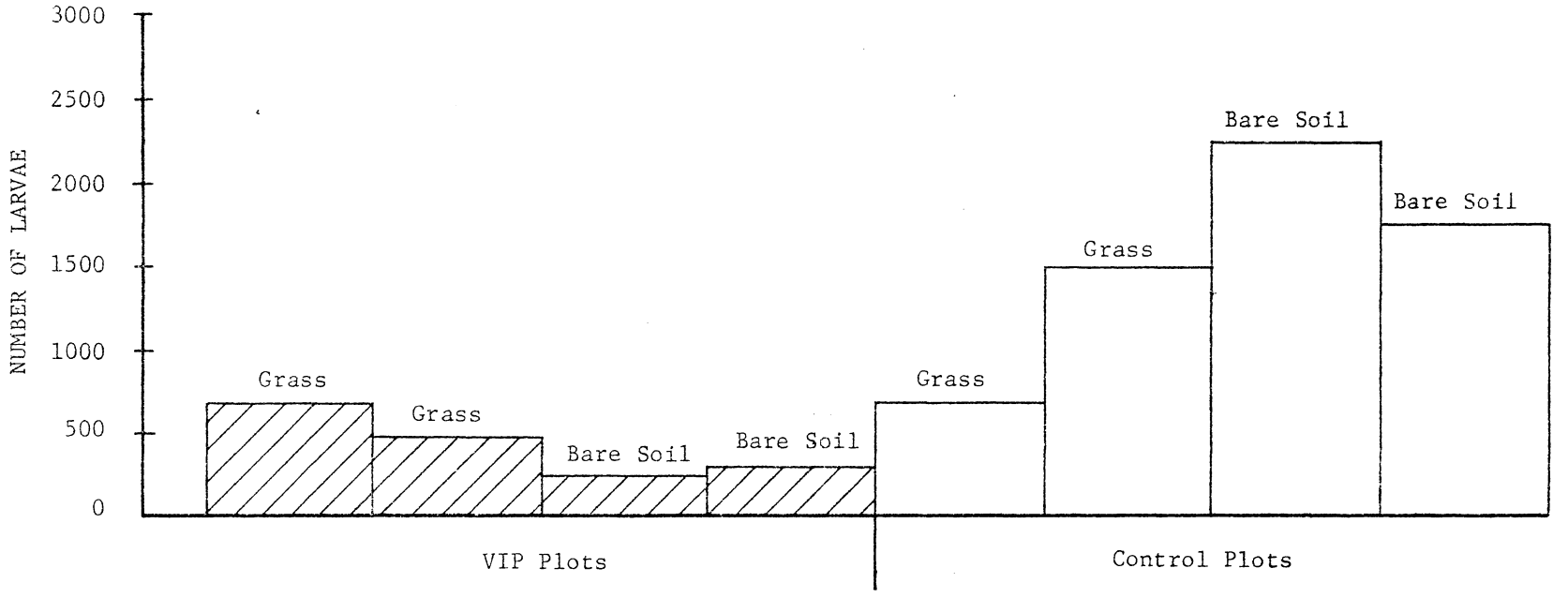


Figure 11. The Number of Ancylostoma caninum Larvae Recovered from Outdoor Grass and Bare Soil Plots Contaminated with 1.5×10^4 Infective Larvae 30 Hours After Treatment (Trial 2)

CHAPTER V

DISCUSSION

In Experiments 1 and 2, larval development was monitored only until it appears all eggs had hatched (1st and 2nd stage). At this point, it was assumed that the larvae which could develop (or survive) to 3rd stage would do so in a maximum of 21 additional days hence.

Due to the simple quantitative technique employed to observe larval development, results can be interpreted only as an estimation of parasitic development. Samples for flotation were taken from the feces in the respective plots, in order to reduce sample contamination with free-living soil and plant nematodes. When the fecal mounds had dispersed, further monitoring of plots was virtually impossible without significant alteration to environmental conditions. Additionally, later in thid study, plot development observations were probably hindered due to lateral migration of larval away from the feces as observed by Arant, et al. (22).

Since development to 1st and 2nd stage larvae was considerably longer than under optimum conditions, weather conditions probably were the major delaying factor. This supposition is based on the observation that the rate of development and survival in Experiments 1 and 2 were quite similar. Although, these experiments were conducted at different times of year, total precipitation and temperature range were quite similar for the two.

In Experiment 1, the plots examined at the larvated egg stage failed to yield larvae. It is possible that no A. caninum larvae had yet hatched or any larvae that had hatched were not yet 3rd stage and were susceptible to the HCl treatment owing to the lack of a protective sheath. Since the hatched larval plots produced the highest yield of larvae, one might speculate that by shortening the period of exposure to the detrimental effect of the environment higher larval yield may be expected. It should be noted that even with these relatively high larval yields than in other plots, no difference between VIP and control plots in this group could be found as was true for the larval egg group, 10 and 21 day post hatch group.

Since strongyle larvae have been shown to survive for up to 10 months in a controlled environment, the rapid decrease of larvae with or without treatment is indicative of adverse environmental factors (12). Further, plots examined in the spring indicate overwintering capability of A. caninum larvae is not likely in Oklahoma.

As in Experiment 1, the extremely low numbers of larvae recovered from all experimental plots (treated and control) in Experiment 2 can be interpreted to be a result of environmental conditions. On day 7, the plots received .88 inches of rainfall in a period of 45 minutes with flooding and possibly overflow. This may have displaced larval over and out of the plots.

Both in Experiment 1 and 2, no statistical difference was found between VIP larvicide treated plots and controls.

It should be noted that parasite recovery from grass and soil determination may be in error due to the technique employed. Although Shorb's technique for the hydrochloric acid has been used by many

workers, it is possible that the HCl is not as selective in killing free-living nematodes as is generally accepted (4)(5)(8). In preliminary studies by the author, all laboratory reared 3rd stage A. caninum larvae tested do survive. However, many free-living nematodes (differentiated from parasitic larvae morphologically) also survived. Additionally it is possible that adverse environmental conditions cause A. caninum to be weakened and thereby killed in the presence of 3% HCl. This could cause decreases in total parasitic larvae counts from each plot.

Experiment 3 was designed to help minimize the adverse effects that Oklahoma environment has on larval development and survival of A. caninum. The results of this experiment shows, quite clearly, that VIP has larvicidal properties, on larvae existing in both grass and bare soil substrates when temperature and humidity are controlled for optimum larval development. Outdoor bare soil, however, was shown consistently to be less favorable for larval survival while indoors this bare soil exhibited essentially no difference from grass plots. This may be interpreted that soil with no vegetation leaves larvae less protected then when grass is present (8).

Larvae on indoor plots generally survived better than those on outdoor plots in both the control or treated groups. This is indictive of the detrimental effect of Oklahoma climate on A. caninum larvae with or without use of VIP larvicide. The overall increase in larval recovery in Experiment 3, may possibly be due to rainfall during this experimental period which prevented larval death from dessication.

The results of all three tests suggest that environmental factors of Oklahoma are detrimental to larvae even during what might be considered optimum conditions for survival, that is April and October.

In determining whether or not using VIP or any larvicide in Oklahoma is advantageous, some appropriate questions should be asked:

1. Is the area to be treated heavily contaminated with A. caninum egg laden feces?
2. Is this contaminated area subject to the full effects of the detrimental environment or are the larvae protected (i.e., is this area shaded or unshaded, moist or dry, etc.)?
3. What is the nature of the contaminated substrate (grass, bare soil, concrete, etc.)?
4. Are the infected animals young puppies or adults? If they are puppies, what role does in utero or cholostral infection have in infection rate?

If the area in question is heavily contaminated, protected from the full effects of the environment and having suitable substrate (e.g., grass), use of the larvicide would seem advantageous. VIP has the advantage over larvicidal borax and sodium chloride in that there was no visible evidence grass was damaged.

In order to accurately determine the development and comparative survival of larvae with or without VIP treatment, one must study the problem as it occurs in nature. Therefore, a primary modification of this research would be to combine the experimental design of these three studies into one comprehensive experiment that would integrate parameters more intimately. Evaluation of the rate of development might be more accurately determined by preparing developmental plots separately from those used

to study survivals. The developmental plots may be a charcoal and feces mixture in sufficient quantity to be sampled daily for the length of the experiment by some reliable counting method (e.g., Stoll method) in order to provide a ratio of each stage of development (23).

Survival recovery determination should also be modified. Preliminary trials with a 3% HCl solution should be conducted until the investigator is convinced of its accuracy. If not, possible alternative methods should be considered. One possible method might be the use of hyperimmune serum. Otto found that serum protein precipitate will adhere to the anal and oral regions of A. caninum larvae (25).

The study of free-living and infective stages of this nematode under natural conditions is extremely difficult due to the large number of factors affecting the organism development and survival. Further research should be directed towards effectively incorporating these factors into one definitive study.

The purpose of using azium was to immunologically compromise the otherwise possibly hookworm resistant dogs by causing a reduction in reducing inflamatory response (26). The fact that no dog received a patent infection may be explained may possible ways:

1. The corticosteroid treatment may not have made the dogs more susceptible to infection (17).
2. Larvae from plots may have lost their capability to infect.
3. The relatively low larval doses administered coupled with the dogs own innate resistance, due to their age and high quality diet, may account for the failure of worms to mature (28).

4. The possibility for migration and storage in the tissue exists since seven of ten dogs were females (29).
5. No parasitic larvae were in fact recovered from the plots.

CHAPTER VI

SUMMARY

Results of three experiments showed that temperature and rainfall, and other climatic stresses in Oklahoma are highly detrimental to *Ancylostoma caninum* free-living larvae. Data from Experiment 1 and 2 could not be statistically analyzed with regard to the comparative efficiency of VIP versus environmental factors in killing larvae. In Experiment 1, data from experimental infection of dogs have no positive results. Therefore, viability of larval (or their presence) was not shown. Data from Experiment 3 showed that VIP has larvicidal capability.

LITERATURE CITED

1. Georgi, J. R. 1974. Parasitology for Veterinarians. W. B. Saunders Co., Philadelphia, 1974:273-274.

2. Stiles, C. W. 1921. "Condensed Hookworm Thermometer." J. Parasitol., 7:192.

3. McCoy, C. R. 1930. "The Influence of Temperature, Hydrogen-Ion Concentration, and Oxygen Tension of the Development of the Eggs and Larvae of the Dog Hookworm Ancylostoma caninum."

4. Levine, N. D. and Anderson, F. L. 1973. "Development and Survival of Trichostrongylus colubriformis on Pasture." J. Parasitol., 59:147-165.

5. Anderson, F. L., et al. 1970. "Survival of Third Stage Trichostrongylus colubriformis Larvae on Pasture." J. Parasitol., 52:209-231.

6. Augustine, D. L. 1923. "Investigations of the Control of Hookworm Disease XXII: Experiments on the Factors of Determining Length of Life of Infective Hookworm Larvae." Am. J. Hyg., 3:420-443.

7. Nicoll, W. 1917. "Observation of the Influence of Salt and Other Agents in Modifying the Larval Development of the Hookworms: Ankylostoma duodenale and Necator americanus. Parasitology, IX:157-189.

8. Mark, D. L. 1975. "Survival of Ancylostoma caninum on Bare Ground, Pea Gravel, and Concrete." Amer. J. Vet. Res., 36:1803-1807.

9. Levine, N. D. 1963. "Weather, Climate and the Bionomics of Ruminant Nematode Larvae." Adv. Vet. Sci., 8:215-261.

10. Beaver, P. C. 1953. "Persistence of Hookworm Larvae in Soil." Amer. J. Trop. Med. Hyg., 2:102-108.

11. Parkin, J. T. 1975. "The Effect of Moisture Stress Upon the Hatching of Nematodirus battus." Parasitology, 71:149-155.

12. Persson, L. 1974. "Studies on the Survival of Eggs and Infective Larvae of Ostertagia ostertagi and Cooperia oncophora in Herbage." Zbl. Vet. Med. Bul., 21:787-798.

13. Mark, D. L. 1975. "Survival of Ancylostoma caninum on Bluegrass Pasture." J. Parasitol., 61:484-488.

14. Underwood, P. C. 1935. "The Use of Saturated Sodium Chloride Solution as a Larvicide in the Control of the Dog Hookworm, Ancylostoma caninum." The N. Amer. Vet., 16:41-44.

15. McCulloch, E. C. 1936. "Disinfection of Kennels." Vet. Med., 31:485-486.

16. Levine, N. D. 1949. "The Effects of Various Compounds Upon Horse Strongyle Larvae in Feces." Amer. J. Vet. Res., 10:233-239.

17. Ackert, J. E. and Ligenzowski, F. L. 1950. "Chemical Control of the Dog Hookworm Ancylostoma caninum." Amer. J. Trop Med., 31:259-266.

18. Hoerlein, B. F. 1950. "The Evaluation of Various Chemical Agents in the Treatment of Soil Infected with Larvae of the Dog Hookworm (Ancylostoma caninum)." The N. Amer. Vet., 31:253-262.

19. Blank, J., et al. 1959. "The Effects of Chemical and Physical Agents on Filariform Larvae of Ancylostoma braziliense." Amer. J. Trop. Med. and Hyg., 8:401-404.

20. Shorb, D. A. 1937. "A Method of Separating Infective Larvae of Haemonchus contortus from Free-Living Nematodes." Proc. Helm. Wash., 54:52.

21. Larsh, J. E., Jr. and Kent, D. E. 1949. "The Effects of Alcohol on Natural and Acquired Immunity of Mice to Infection with T. spiralis." J. Parasitol., 35:45-53.

22. Arant, F. S. and Knapp, R. 1949. "A Simple Method of Rearing and Mounting Hookworm Larvae." Science, 75:495-496.

23. Augustine, D. L. 1922. "Investigations on the Control of Hookworm Disease x Experiments on the Length of Life of Hookworm Larvae." Amer. J. Hyg., 2:177-187.

24. Stoll, N. R. 1922. "Investigations on the Control of Hookworm Disease XV. An Effective Method of Counting Hookworm Eggs in Feces." Amer. J. Hyg., 3:59-70.

25. Otto, G. F. 1948. "Immunity Against Cainine Hookworm Disease." Vet. Med., 43:180-191.

26. McKee, P. A. 1970. "Effects of the Spleen on Canine Factor VIII Levels." J. Lab. & Clinical Med., 75:391-402.

27. Miller, T. A. 1966. "The Effect of a Synthetic Corticosteroid upon the Susceptibility of Dogs to Infection with the Human Hookworm, Necator americanus." J. Parasitol., 52:285-290.

28. McCoy, O. R. 1931. "Immunity Reactions of the Dog Against Hookworm (Ancylostoma caninum) Under Conditions of Repeated Infection." Amer. J. Hyg., 14:268-303.

29. Foster. A. O. 1933. "Prenatal Infection with the Dog Hookworm, Ancylostoma caninum." J. Parasitol., 19:112-120.

VITA

Lawrence David Shamis

Candidate for the Degree of

Master of Science

Thesis: A STUDY OF THE EFFECT OF SOME OKLAHOMA ENVIRONMENTAL FACTORS OF THE LARVICIDAL ABILITY OF DIMETHYL PHOSPHATE OF α-METHYLBENZYL 3-HYDROXY-CIS-CROTONATE 2,2-DICHLOROVINYL PHOSPHATE (VIP-DICHLORVOS) ON THIRD STAGE *ANCYLOSTOMA CANINUM* (ERCOLANI, 1859) LARVAE

Major Field: Veterinary Parasitology and Public Health

Biographical:

Personal Data: Born in Bronxville, New York, December 2, 1948; the son of Mr. and Mrs. Jack Shamis.

Education: Graduated from Great Neck South High School in May, 1967; received Bachelor of Science Degree in Animal Health Science from the University of Arizona in Tucson in 1974.

Professional Experience: Research assistant in the Department of Veterinary Parasitology, Microbiology, and Public Health, Oklahoma State University, 1974-1977.

Professional Associations: American Society of Parasitologists; American Association of Veterinary Parasitologists; American Society for Microbiology, American Society for the Advancement of Science.