THE FINAL REPORT OF THE CORN CYST NEMATODE SURVEY IN VIRGINIA

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INTRODUCTION

The corn cyst nematode, *Heterodera zeae* Koshy, Swarup, and Sethi, 1970, was first described parasitizing corn in India in 1970 (Koshy *et al.*, 1970). Thereafter, *H. zeae* was detected in Egypt in 1978 (Oteifa in USDA, 1986) and in Pakistan (Maqbool, 1981) and the United States (Sardanelli *et al.*, 1981) in 1981. The corn cyst nematode has been reported to be widespread in the corn growing areas of India (Koshy and Swarup, 1971) and Egypt (Aboul-Eid and Ghorab, 1981). In the United States, however, *H. zeae* was found only in four counties in Maryland (Roth, unpubl.). The corn cyst nematode was recently detected for the first time in Cumberland County, Va., approximately 170 miles from the closest known occurence of *H. zeae* in Maryland (Eisenback *et al.*, 1992).

Heterodera zeae parasitizes corn and several other graminaceous species including cereal crops and weeds (Koshy *et al.*, 1970; Srivastava and Swarup, 1975; Verma and Yadav, 1978; Aboul-Eid and Ghorab, 1981; Ringer *et al.*, 1987), although reports on its pathogenicity to flint corn, oat, wheat, rice, and sorghum are contradictory. Differences in host reaction is attributed to genetic differences in the population of the host or parasite, or to variation in the environmental conditions under which the tests were conducted.

Heterodera zeae was shown to be pathogenic to corn under greenhouse conditions in Egypt (Aboul-Eid and Ghorab, 1981) and India (Srivastava and Sethi, 1984). It attacks the root system of corn plants resulting in poor root development, stunting, pale narrow leaves, and retardation of leaf emergence. The corn cyst nematode is considered to pose an economic threat to the corn production in India (Koshy and Swarup, 1971), Egypt (Aboul-Eid and Ghorab, 1981) and in corn-growing regions of the United States that are warmer than Maryland (Hutzell and Krusberg, 1990).

The corn cyst nematode has been suggested to be responsible for yield reduction of corn (Sardanelli *et al.*, 1981; Eisenback *et al.*, 1992); however, there are no studies on the effect of this nematode on corn production in the field. The corn cyst nematode is not limited by any particular soil texture, although it prefers moderately light soil for reproduction (Srivastava and Sethi, 1984) and requires high soil temperatures for development and reproduction (Bajaj and Bhatti, 1984; Hutzell and Krusberg, 1990).

The optimum temperature for development of *H. zeae* is 33 C; one life cycle is completed in 15-18 days, but at 25 C the life cycle is increased to 42 days. Under controlled conditions fastest development, highest reproduction, and heaviest infection occurred between 32 and 36 C (Bajaj *et al.*, 1986; Hutzell and Krusberg, 1990). Penetration was

severely retarded at 20 C and development beyond the second-stage juveniles was arrested at 15 C (Bajaj *et al.*, 1986; Hutzell and Krusberg, 1990); however, corn cyst nematodes overwinter as cysts in the field without detectable mortality (Krusberg and Sardanelli, 1989). The corn cyst nematode is able to complete 7 to 8 generations in one growing season in India (Srivastava and Sethi, 1986). The temperature requirements of the corn cyst nematode is probably responsible for the low population densities detected in most fields in Maryland (Hutzell and Krusberg, 1990).

The objective of this study was to determine the distribution of *H. zeae* in Virginia.

MATERIAL AND METHODS

Sample collection: Two schemes were used to collect soil samples from the area near to the site where *H. zeae* was first detected.

(a) Growers from Cumberland and surrounding counties near the site where the corn cyst nematode was first discovered collected 220 soil samples from fields that were mainly utilized for corn and/or pasture (Table 1). The samples were collected between December, 1993 and July, 1994.

Table 1: Distribution by county and crop of soil samples collected by the growers.

Crop	Corn	Pasture	Small grains	Soybean	Vegetables	Tobacco	Not listed	**Total**
County								
Amelia	6	3	8	18	-	-	-	**35**
Buckingham	-	14	-	-	-	-	-	**14**
Chesterfield	1	4	-	1	-	-	-	**6**
Cumberland	29	108	12	4	3	5	2	**163**
Powhatan	-	-	-	-	1	-	1	**2**
Total	36	129	20	23	4	5	3	**220**

(b) A target survey was conducted in three phases. The results of each phase were used to proceed to the next phase. In the first phase samples were collected from fields with corn debris on the surface or emerging corn plants from fields withing a two and a half mile radius of the field on Forkland Farm that originally was found to be infested with corn cyst nematades. This circle included parts of Cumberland, Amelia, and Prince Edward Counties.

In the second phase samples were collected from fields with corn debris on the surface or emerging corn plants from fields in the flood plain of the Appomattox River, from its origin in Appomattox County to its mouth in Prince George County.

In the third and phase samples were collected from fields farmed by Forkland Farm including all fields on the homeplace, as well as all fields that were leased by the farm within the last five years. Fields were mainly planted to corn, pasture, or small grain.

The distribution of samples by county is shown in Table 2. The latitude and longitude of each sample was determined by a global positioner. Samples were collected between April and June, 1994.

Table 2: Distribution by county of the soil samples collected during the target survey.

County	5-mile radius	Outside 5-mi radius	**Total**
Cumberland	282	34	**315**
Prince Edward	6	2	**8**
Amelia	5	13	**18**
Appomattox	-	13	**13**
Powhattan	-	4	**4**
Dinwiddie	-	1	**1**
Prince George	-	5	**5**
Charles City	-	2	**2**
Total	293	74	**367**

Sampling: Each soil sample was collected from 20 subsamples of each site of 5 acres each. Fields larger than 5 acres were divided into 5-acre or smaller units. Subsamples of the upper 20 cm of soil were taken with a shovel next to corn stubs or growing corn plants whenever possible and thoroughly mixed in a bucket. Samples were placed in plastic bags, labeled, and transported back to the laboratory for analysis. Growers were instructed to use the same procedure for sample collection which was often supervised by a county agent.

Extraction: Nematodes were extracted from the soil with a semi-automatic elutriator and centrifugal flotation. Five hundred ml of soil were used to extract nematodes with 25 and 60 mesh sieves. The nematodes and debris caught on the 60-mesh sieve were collected and processed by centrifugal flotation in an aqueous solution of 50% sucrose by weight.

Identification and quantification: *H. zeae* was identified by the morphology of the cyst, vulval cone, and stylet and tail of second-stage juveniles (Fig. 1). The number of cysts per sample was expressed as cysts per 500 cc.

Samples that contained few cysts of *Heterodera* spp. were used for bioassays on corn plants in the greenhouse because accurate identification to species was not always possible by morphology alone. The bioassays were conducted by mixing soil from the sample with steam-sterilized soil in a 5-cm diameter clay pot. The pots were planted with four seeds that were thinned to one after emergence. The pots were placed on a thermal mat that maintained the soil temperature at 33 C. The plants were harvested after a 4-6 months and the cysts were extracted by sieving and centrifugal flotation.

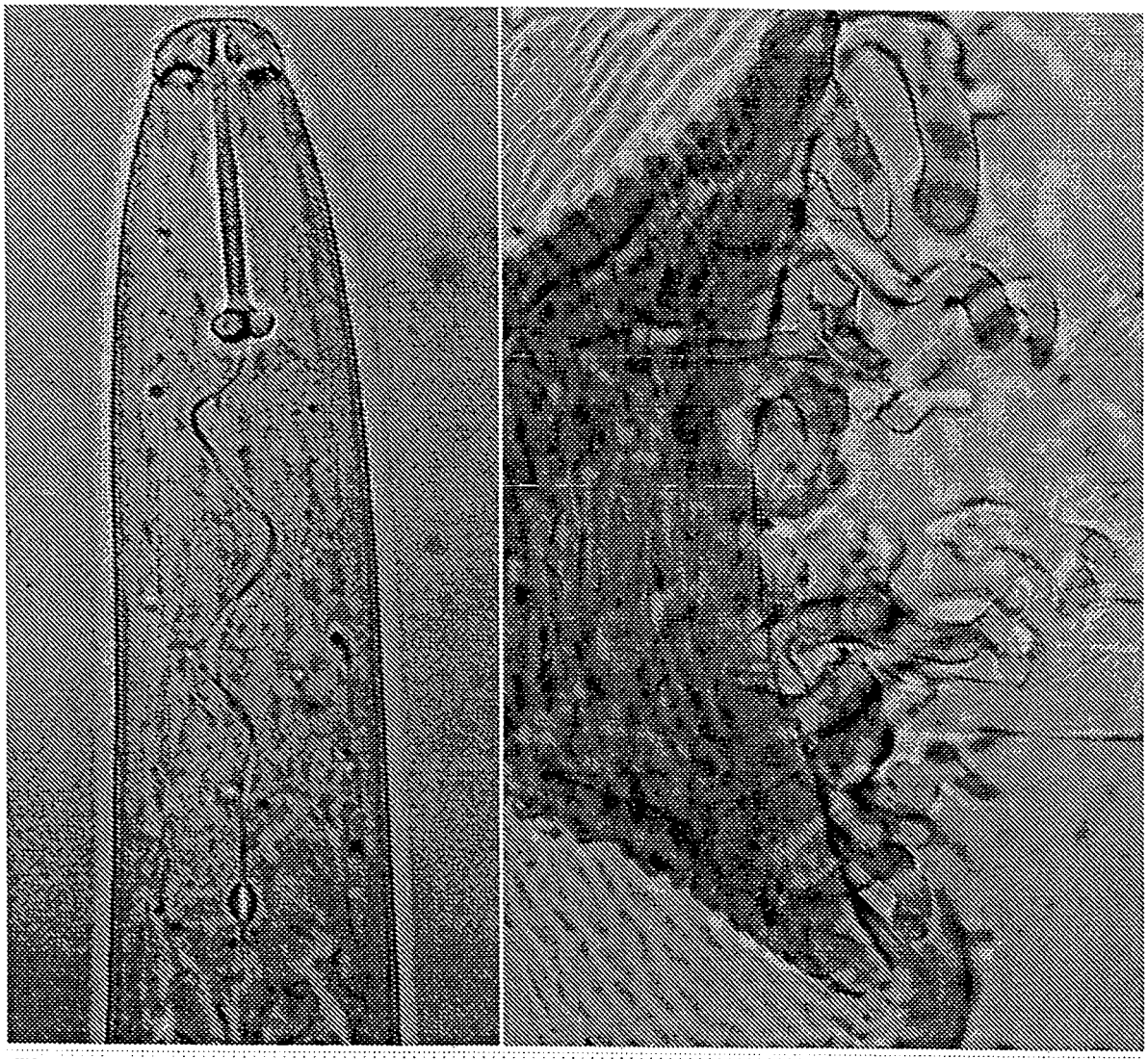

Figure 2. Light micrographs of the anterior end of a second-stage juvenile and the lateral view of the vulval cyst cone of corn cyst nematode.

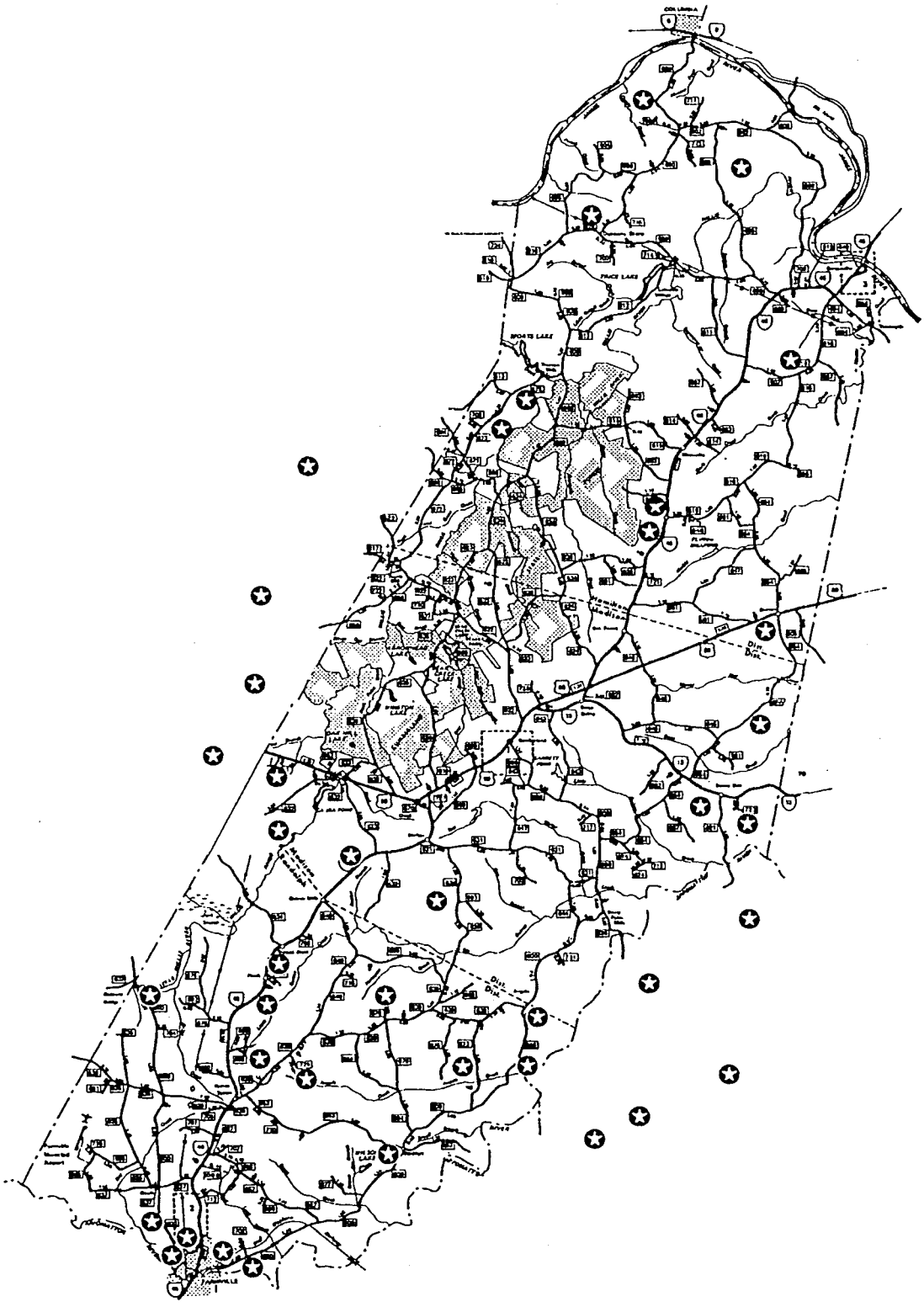

Figure 3. Survey sites in Cumberland and surrounding counties.

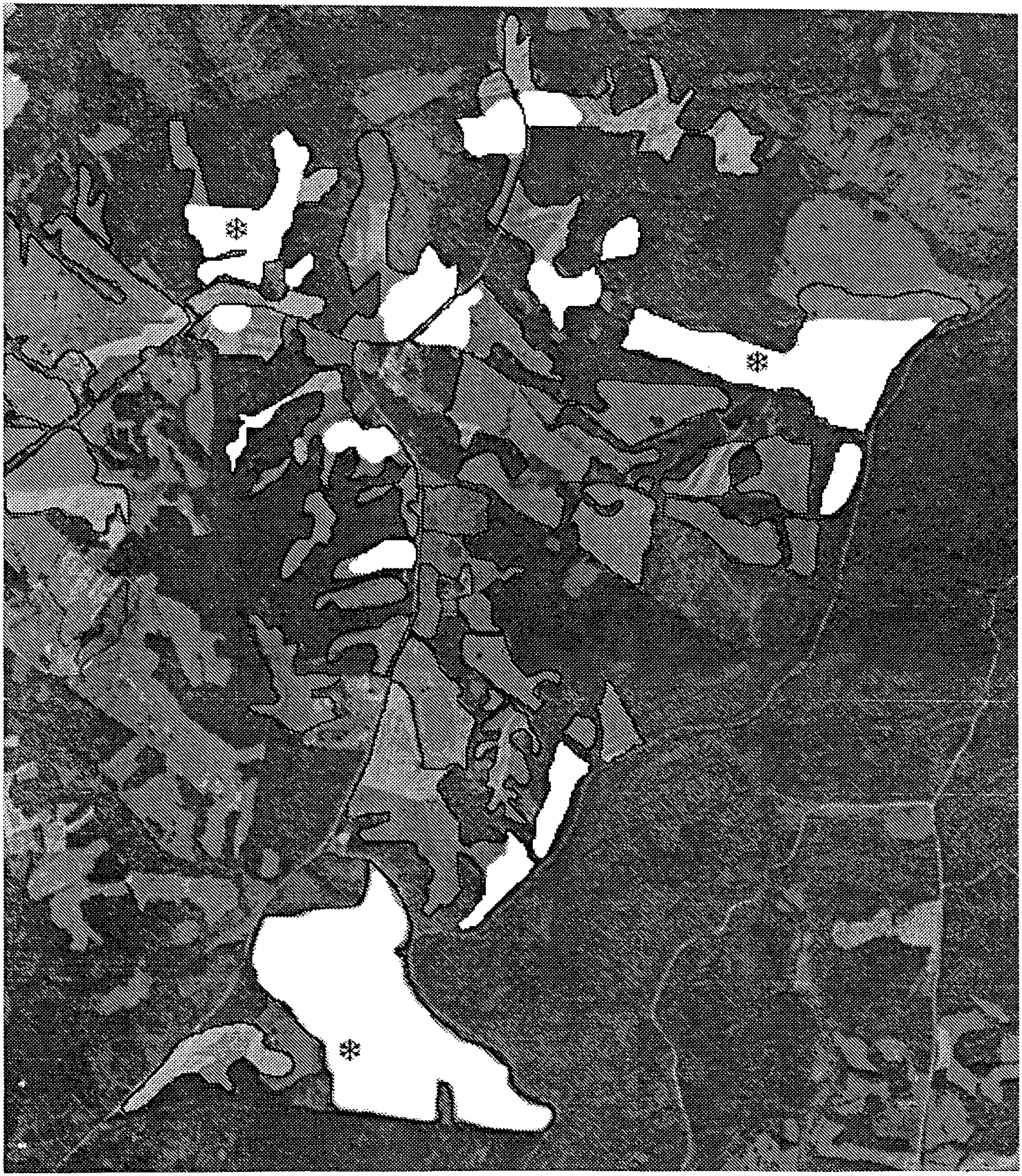

Figure 4. Known distribution of corn cyst nematode in Virginia. Outlined fields were included in the survey. Fields highlighted in yellow were positive for the nematode. ❄=fields with the highest levels of infestation.

RESULTS

Approximately 3,930 acres were surveyed for the corn cyst nematode, 1,100 acres by the growers and 1,830 acres in the target survey. The approximate location of the samples taken by the growers is shown in Fig. 2. The locations of the samples taken in the target survey were within a two and a half mile radius of the original field, and from the flood plain of the Appomattox River from its origin to its mouth and beyond on the James River.

Only 2 of 220 samples collected by growers were infested with *H. zeae*. Both samples were collected from fields planted with a mixture of orchard grass and clover on the Forkland Farm. Twenty additional samples were infested with lemon-shaped cyst nematodes and bioassays confirmed that they were not corn cyst nematodes. Only the samples from Forkland Farm contained cyst nematodes that reproduced on corn.

Of the remaining 316 samples, only those collected from Forkland Farm were infested with corn cyst (Fig. 4). Seventy-five samples representing 375 acres were infested, all within 3 miles of the original find. All of the infested fields were located between latitude N 37°22'13.6" and 37°24'44.2" and between longitude W 78°12'58.6" and 78°14'58.4". All infested sites were farmed by Mr. Hazlegrove, owner of Forkland Farm and were located in a 3 by 2.5 mile strip on the northern side of the Appomattox River.

The positive 75 samples contained 1-32 cysts/500 cc of soil and only three possessed more than 20 cysts/500 cc of soil (Fig. 5). The sample with the highest number of cyst yielded 32 cysts/500 cc of soil and was collected in a field near the mouth of Angola Creek; the second highest, 29, was collected close to Guinea Rd. at its intersection with River Rd.; and the third highest, 26, was collected from the field where the corn cyst nematode was originally detected.

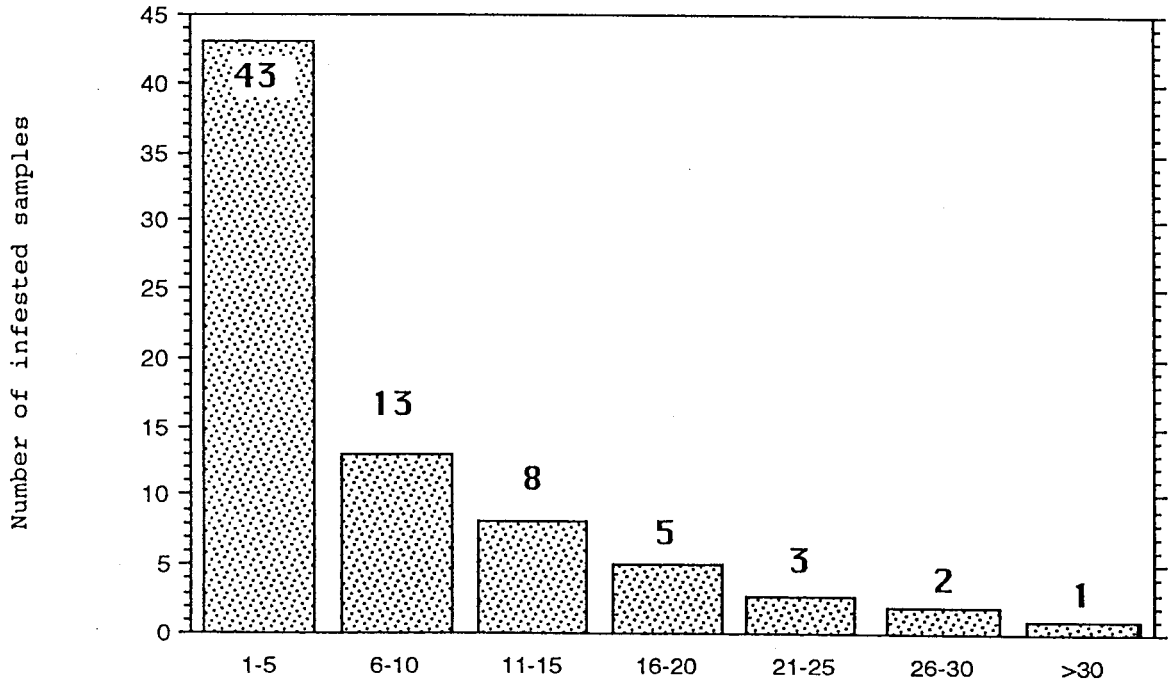

Figure 5. Concentration levels of cysts of *H. zeae* detected in the survey.

None of the 55 samples from fields along the Appomattox River from its origin in Appomattox County to its mouth in Prince George County, including some fields on the James River contained *H. zeae*. Likewise, none of the fields that Mr. Hazelgrove leased in the county were found to be infested with the corn cyst nematode. All of the positive finds were on or contiguous to the Forkland Farm and from corn fields that were farmed by Mr. Hazelgrove.

DISCUSSION

The distribution of *H. zeae* in Virginia appears to be restricted to a 3 by 2.5 mile strip of land in Cumberland County along the Appomattox River, on both sides of River Road, and both sides of Guinea Road, all property under the management of Mr. Hazlegrove (Fig. 4, 6). Many infested fields contained sandy soils, but fields with clay soil were also infested. Higher populations of corn cyst nematode were found in sandy soils as noted by Srivastava and Sethi (1984) who detected higher reproduction in moderately light soils.

No evidence was found that this nematode had been spread through the flooding of the Appomattox River. Likewise, results of this survey did not confirm long distance spread by the movement of soil, because none of the fields leased by Mr. Hazelgrove were found to be infested.

The populations of corn cyst nematode were generally very low. This survey was conducted in the spring when corn was not growing or in early stages of development, and could be responsible for the low populations of *H. zeae*. Also, the lower than optimal temperatures that occur in Cumberland County may be responsible for these low populations. However, if this was a recent introduction, the nematode population may be low because the nematodes have not increased to their potential level.

The mechanism by which this nematode has become established in Cumberland County, 170 miles from the nearest infested field in Maryland, is unknown. The spread on the Forkland Farm is probably from the movement of soil and soil attached to farm machinery (Fig. 6). The lack of detection of *H. zeae* in other fields leased by Mr. Hazlegrove outside the homeplace may be because the amount of contaminated soil adhereing to farm machinery is very low and transport of cysts to these fields has not occurred. It is also possible that these fields have been contaminated, but the populations remain below detectable levels.

This nematode is likely to spread to other farms surrounding Forkland Farm. Movement of infested soil from farming activities, Department of Transportation activities, and natural soil movement through erosion and flooding are likely (Fig. 6). Fortunately, it appears that the temperature requirements for the completion of the life cycle of this nematode prevent it from posing a serious threat to corn production in Virginia.

Mr. Hazelgrove and other managers of Forkland Farms can limit the spread of the nematodes to surrounding farms by minimizing the movement of soil including that found on farm equipment. Likewise, the Virginia Department of Transportation can also prevent the spread of this nematode by understanding the potential threat that this nematode poses to farm land and realizing the impact that the movement of contaminated soil in the normal maintenance of the roads that bisect contaninated fields on River Road, Angola Road and others.

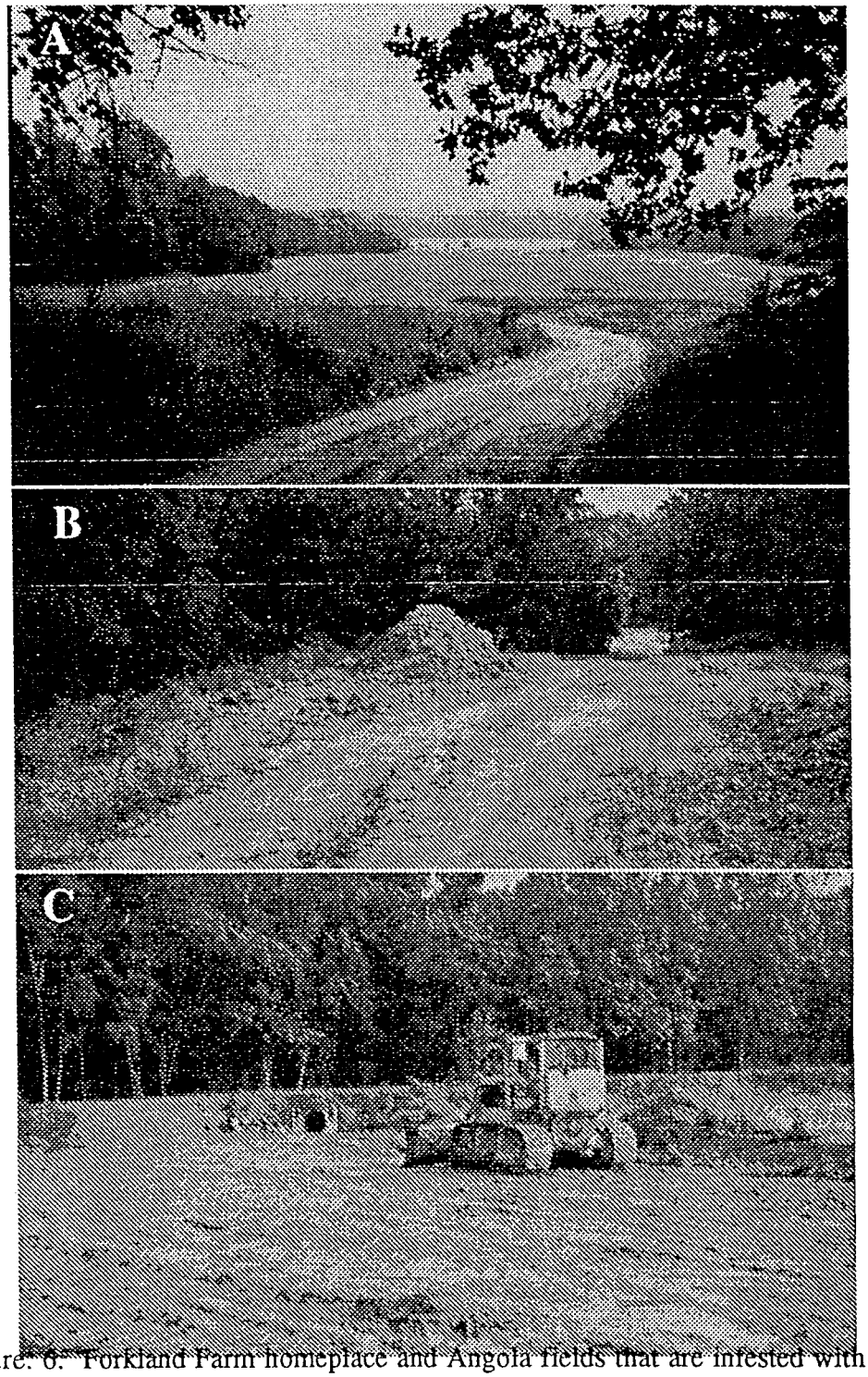

Figure 6. Forkland Farm homeplace and Angola fields that are infested with the corn cyst nematode. A) The field where the nematode was found originally. B) A pile of soil illustrating the movement of soil. C) A bulldozer moving soil around in a field that was later moved to other fields.

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Signature Responding State 

Commissioner, Virginia Department of Agriculture
and Consumer Services

Date 10-12-93

Corn Cyst Nematode Survey in Virginia: An Interim Report

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INTRODUCTION

The corn cyst nematode (CCN), *Heterodera zeae* Koshy, Swarup, and Sethi, is a parasite of corn and was described first in India in 1970. Thereafter, the pest was detected in Egypt (1978), Pakistan and the United States (1981). CCN is widespread in corn production areas of India and Egypt, but had been restricted to four counties in Maryland from the time of detection in 1981. A second incidence of CCN in the United States has been documented with the confirmation of *H. zeae* in Cumberland County, Virginia. The nematode was detected during a routine nematode assay on soil from a farmer who was experiencing yield reductions. No research has been conducted to study the effect of CCN on corn yield.

H. zeae parasitizes corn and other gramineaceous hosts including cereal crops and weeds. There have been contradictory reports on the pathogenicity of CCN on certain crops such as flint corn, oat, wheat, rice and sorghum. Differences in host reactions may be attributed to genetic differences in the population of either host or parasite, or to variations in the environmental conditions. *H. zeae* was shown to be pathogenic on corn grown under greenhouse conditions in Egypt and India.

The nematode, which attacks the root system, causes poor root development, stunting, pale narrow leaves, and retardation of leaf emergence. CCN has been considered a serious economic pest to the corn production areas in Egypt and India, and poses a potential threat to areas of the United States that have longer and hotter growing conditions than Maryland.

CCN is not limited to a particular soil type or texture, although moderately light soils facilitate reproduction. High soil temperatures are required for development and reproduction. The life cycle takes 15-18 days at 33°C, which appears to be the optimum for development, but takes 42 days at 25°C. Under controlled conditions, the fastest development, higher reproductive rate, and heaviest infection occurred between 32° and 36° C. Root penetration was restricted severely at 20°C and development beyond the second stage juvenile was arrested at 15°C.

Temperature differences among areas in the United States and areas in India or Egypt may partially explain differences in distribution and population densities. In India where growing conditions remain hotter longer, the nematode can complete 7 or 8 generations in one season, thereby achieving greater population densities. In the United States, the apparent restricted distribution and low population levels detected in most fields in Maryland and Virginia may be due to less than optimal temperatures.

The objective of this study is to determine the distribution of *H. zeae* in Virginia.

MATERIAL AND METHODS

Sample collection: Two schemes were used to collect soil samples from the area near the site where *H. zeae* was first detected.

(a) Growers from Cumberland County and counties near the site where CCN was discovered collected 218 soil samples from fields where corn and pastures were grown (Table 1). Samples were collected between December, 1993 and May, 1994.

Table 1: Distribution by county and crop of soil samples collected by the growers.

Crop / County	Corn	Pasture	Small grains	Soybean	Vegetables	Tobacco	Not listed	Total
Amelia	6	3	8	18	-	-	-	35
Buckingham	-	14	-	-	-	-	-	14
Chesterfield	1	4	-	1	-	-	-	6
Cumberland	29	108	12	4	2	5	2	162
Powhatan	-	-	-	-	1	-	-	1
Total	36	129	20	23	3	5	2	218

(b) A target survey was conducted in two phases from fields previously planted with corn. In the first phase, soil samples were collected in a 5-mile radius from the site where the CCN was first detected. This area included parts of Cumberland, Amelia, and Prince Edward Counties where 100, 5, and 6 soil samples were collected, respectively. The distribution of the collected samples by county is shown in Table 2. Based on results, a second phase of the survey was conducted in corn fields located along the Appomattox River from Appomattox County down river to Prince George County (Table 2). Latitude and longitude of each site of collection was determined by global positioning system satellite technology. Samples were collected between April and May, 1994.

Table 2: Distribution by county of the soil samples collected during the target survey.

County	5-mile radius	Outside 5-mi radius	Total
Cumberland	100	15	115
Prince Edward	6	2	8
Amelia	5	13	18
Appomattox	-	13	13
Powhatan	-	4	4
Dinwiddie	-	1	1
Prince George	-	5	5
Charles City	-	2	2
Total	111	55	166

Sampling: Each soil sample consisted of 20 subsamples from each site that represented 4 acres. Fields larger than 4 acres were divided into 4-acre units. Subsamples of the upper 20 cm of soil were taken with a shovel and thoroughly mixed in a bucket. Samples were placed in plastic bags, labeled, and transported back to the laboratory for analysis. Growers were instructed to use the same procedure for sample collection.

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Extraction: Nematodes were extracted from the soil by a semi-automatic elutriator and centrifugal flotation. Two hundred fifty and 500 cc of soil were used to extract nematodes from the samples collected by the growers and from the five mile radius area, respectively. Sieves of 25 and 60 mesh were used during the extraction. Nematodes and debris from the 60-mesh sieves were collected and processed by centrifugal flotation using 50% sucrose by weight.

Identification and quantification: *H. zeae* was identified based on morphology of the overall cyst, vulval cone, and stylet and tail of second-stage juveniles. Number of cysts per sample was determined and expressed as cysts per 500 cc. Bioassays were conducted when the small number of cysts did not allow an accurate identification. The soil sample was mixed with sterilized soil and placed in a 5 cm diameter pot. Four corn seeds were planted per pot and thinned to one after emergence. Pots were placed on a thermal mat at 33° C and harvested after a minimum of 3 months.

RESULTS

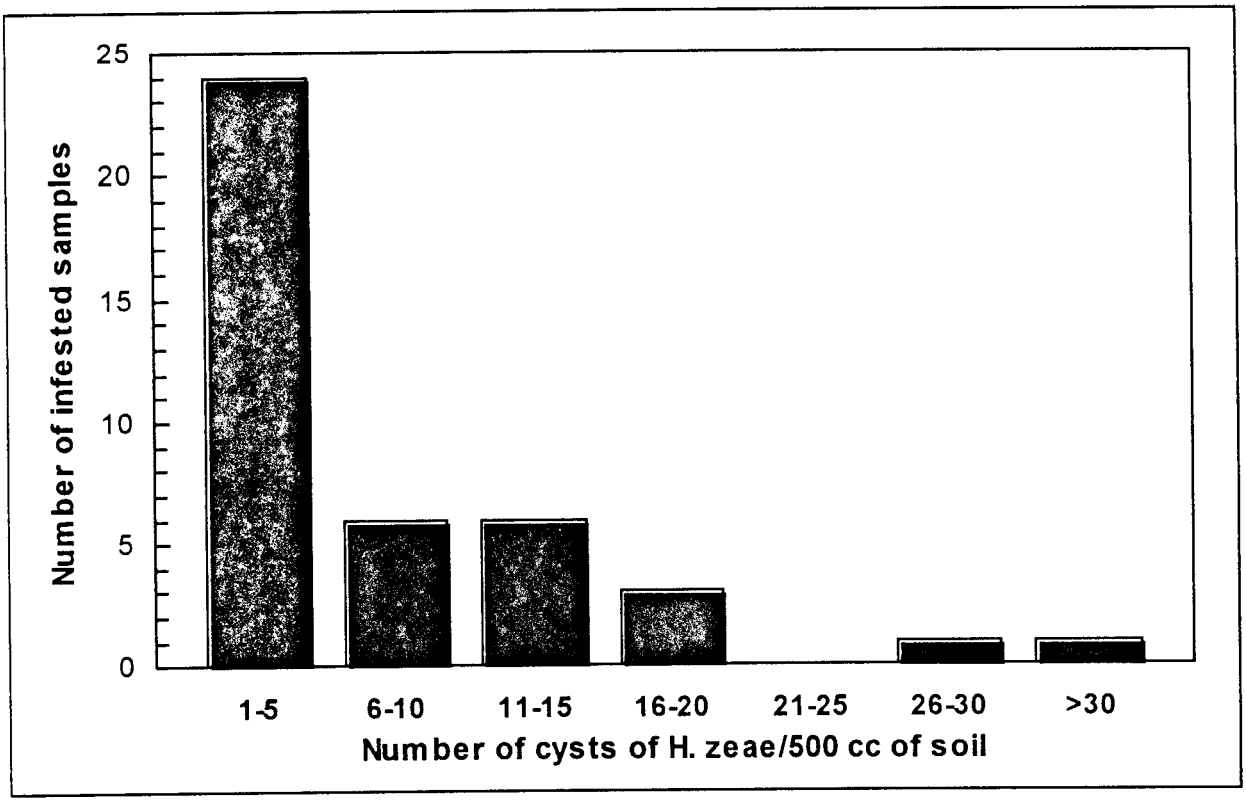
Approximately, 1600 acres have been surveyed for CCN thus far; 872 acres by growers and 672 acres in the target survey. Two of 218 samples collected by growers were infested with *H. zeae*. Both samples were collected from fields planted with orchard grass and clover at Forkland Farm where CCN was first discovered in Virginia. Fifteen samples were infested with *Heterodera* spp. and bioassays are underway to confirm their identity.

Thirty-nine of 111 samples were infested within the 5-mile radius of the site where CCN was originally detected. Sites were located between latitude N 37°22' 13.6" and 37°24' 42.0" and between longitude W 78°14' 45.8" and 78°12' 58.6," and were farmed by Mr. Hazlegrove, owner of Forkland Farm. All positive samples were located within a 1 mile by 2.5 mile strip on the northern side of the Appomattox River and, except for 2 positive samples from red clay soils, all were found in sandy soil. Fig. 1 (attached).

Since all infested sites were in or adjacent to bottom land, a second phase of the survey was extended to fields along the Appomattox River from its origin in Appomattox County to its mouth in Prince George County; some fields on the James River were included. *H. zeae* was not detected in any of the 55 samples collected in the second phase of this survey.

The 41 infested samples contained 1 to 32 cysts/500 cc of soil and only two had more than 20 cysts/500 cc of soil (Fig. 2). The highest number of cysts recovered was 32 cysts/500 cc of soil which was collected from a field near the mouth of Angola Creek emptying into the Appomattox River. The second highest number was 26 cysts/500 cc of soil which was collected from the field where the corn cyst nematode was originally detected in Virginia.

Figure 2: Distribution of infested samples with different concentrations of cysts of *H. zeae*.



The distribution of *H. zeae* in Virginia appears to be restricted to a 1 mile by 2.5 mile strip of land in Cumberland County between the Appomattox River and River Road (state route 600), located from latitude N 37°22'13.6" to 37°24'42.0" and from longitude W 78°14'45.8" to 78°12'58.6." Infested fields are mostly aggregated, bottom land sandy soils. Higher populations of CCN were found in sandy soils. Only two clay fields were infested.

No evidence was found that this nematode had been spread by the flooding of the Appomattox River. The first two phases of this survey were conducted in the early spring and corn was not growing or just emerging.

All infested fields detected are under Mr. Hazlegrove's management. The next phase of this survey will cover all fields that he farms, in addition to other corn fields located along the Angola Creek, since the highest infested field was found adjacent to its mouth into the Appomattox River.

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COOPERATIVE AGREEMENT
BETWEEN
THE PEST CONTROL COMPACT
AND
Virginia Department of Agriculture and Consumer Services
(Responding State)

The principal parties to this Cooperative Agreement are the Interstate Pest Control Compact, hereinafter called the Compact, and the VA Dept of Agric & Consumer Srvs hereinafter called the Responding State.

The purpose of this Cooperative Agreement is to stipulate the general conditions under which the Compact will provide funds to the Responding State to finance other than normal pest control operations, hereinafter called Project, as approved by the Compact Governing Board. Such Project will be provided to the Responding State as an approved "Request for Financial Assistance from the Pest Control Insurance Fund," hereinafter called Request.

The cooperation shall be conducted consistent with the Compact enabling legislation, as adopted by member states, and the Bylaws of the Compact Insurance Fund and with all applicable statutes and regulations of the Responding State.

A. The Compact Agrees:

1. To provide funds in the amount of $ 20,000 to the Responding State upon timely and satisfactory completion of the Project as outlined in the Request, or upon satisfactory evidence that expenses have been incurred on account of measures taken toward Project completion.

2. To furnish the services of the Compact Executive Director whose duties shall include coordinating activities relative to this Cooperative Agreement.

3. To furnish the services of the Compact Technical Committee for advisory purposes, as mutually agreed, or for Project evaluation and monitoring.

B. The Responding State Agrees:

1. To provide necessary resources to perform Project activities as outlined in the Request in an expeditious and efficient manner.

2. To submit a progress report on Project activities to the Compact Executive Director by 3/28/94 (date).

3. To submit a final report, to include evidence of satisfactory and timely completion of the Project and including a detailed financial statement of funds expended, to the Compact Executive Director by 10/31/94 (date).

4. To cooperate fully with the Compact Technical Committee in any evaluation or monitoring of the Project, either during progress or after completion.

5. To maintain pest control and eradication activities of interstate significance at a level that would be reasonable in the absence of the Project.

6. To meet emergency outbreaks or infestations of interstate significance to no less an extent than would have been done in the absence of this Project.

C. It is Mutually Understood and Agreed:

1. That the cooperating parties may mutually agree to minor adjustments in Project details as outlined in the Request, consistent with Project objectives.

2. This agreement shall become effective upon date of final signature and shall continue until 9/30/94 (date), unless amended by mutual agreement of both parties.