2001 FHP TECHNOLOGY DEVELOPMENT PROJECT PROGRESS REPORT

PROJECT STATUS: Continuing.

PROJECT NUMBER: R8-2001-01

PROJECT TITLE: Removing an Imported Predator of Hemlock Woolly Adelgid From Quarantine and Evaluating its Effectiveness as a Biological Control Agent in the Field

SUBJECT: Biological Control, Invasive Species, Organism Biology, Rearing Techniques

PROJECT OBJECTIVES:

1. To evaluate the efficacy of *Laricobius nigrinus* Fender as a predator of hemlock woolly adelgid (HWA) under field conditions:

   a. Evaluate the success in overwintering and establishment of *L. nigrinus* in eastern hemlock forests infested with HWA.

   b. Determine the potential geographic range for *L. nigrinus* as a predator for HWA.

2. Improve rearing techniques for *L. nigrinus* in an effort to move toward mass rearing.

BRIEF DESCRIPTION OF PROJECT: The project has two major facets:

1. We have received permission to release *Laricobius nigrinus* from quarantine and study it in the field. Experiments will be conducted to determine if the beetle can survive and become established on HWA in eastern North America. In addition, it will be necessary to quantify the impact of the predator on HWA populations. Short-term studies include caged experiments and long-term studies involve open field releases.

2. Large numbers of *L. nigrinus* will be needed for field releases and evaluation. Experiments will be conducted on laboratory conditions necessary for successful rearing of the beetle. The aim is to develop procedures for its efficient reproduction. Efforts will focus on composition and moisture of the substrate that the beetle uses for pupation and overwintering. Different soil and peat mixtures will be evaluated. The optimal moisture content and techniques for its maintenance will be studied. Various densities of larvae on HWA-infested hemlock will be evaluated to minimize % mortality of developing *L. nigrinus* larvae.

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PRINCIPAL INVESTIGATORS:

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L. T. Kok, Dept. of Entomology, Virginia Tech.

COOPERATORS:

Lee Humble, Canadian Forest Service, PFC.
Tim Tigner, Virginia Department of Forestry
Richard Evans , Delaware Water Gap, NPS

ACCOMPLISHMENTS AND RESULTS FROM THIS PAST YEAR:

A M.S. student (Ashley Lamb) enrolled at Virginia Tech in August, 2000 and will be conducting the studies necessary to achieve the first phase of the project's objectives.

Objective

1. Field Evaluation

Methods
A caged field study was conducted at 3 sites from February through June, 2001. The sites were located at Poverty Creek, Big Stoney Creek, and Mt. Lake Nature Conservancy, VA. Two trees at each site were chosen and the total numbers of adelgids were counted on 27 branches of each tree. Each branch was then randomly assigned one of three treatments: caged branches with 2 female adult *L. nigrinus*, caged branches without predators, and uncaged branches without predators. The latter two treatments served as controls.

One replicate of each treatment was removed from each tree twice a month, and the total number of surviving predators, both adults and progeny, were tallied. In addition, the total number of surviving adelgids on each branch was determined.

Results
L. nigrinus adults survived the winter and were reproductive from February through March of the study period (Figure 1). Adult survival declined over time, primarily due to a shortage of prey inside the cages. Adult *L. nigrinus* oviposited throughout March and April, producing a maximum of 41 progeny per beetle.

The overall average of adelgid mortality on branches exposed to *L. nigrinus* was significantly higher than the overall adelgid mortality on the control branches (Figure 2).

It is likely that *L. nigrinus* will survive longer, produce a greater number of progeny, and consume more adelgids if a sufficient amount of quality prey was present. This will be investigated further during the 2nd year of the field evaluation (fall-winter 2001-2002).

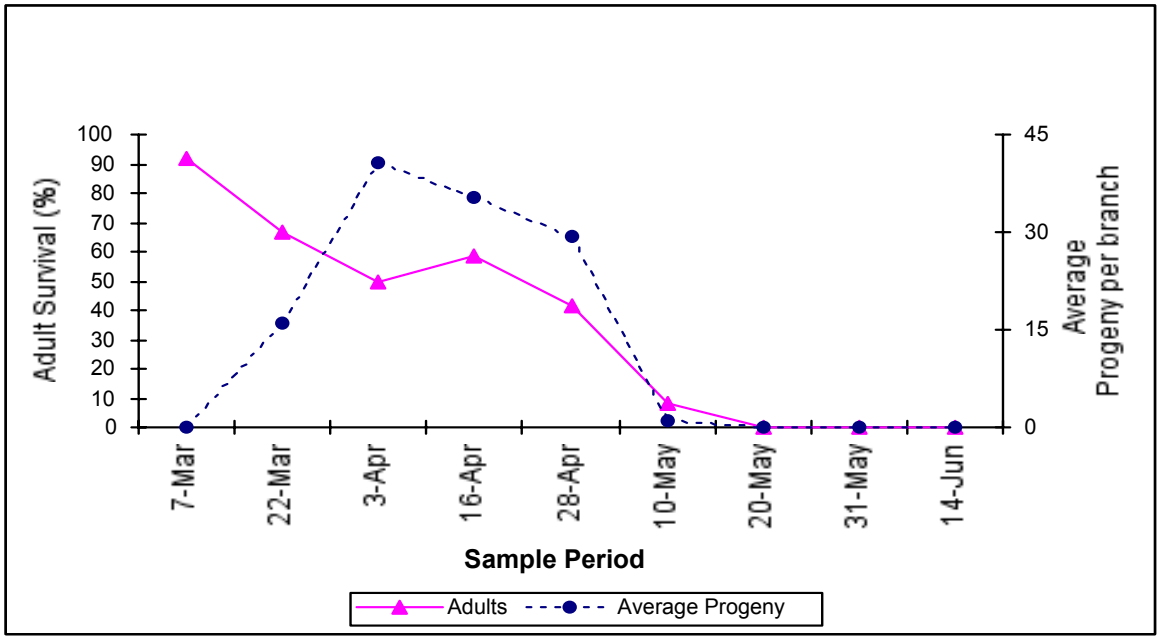

Figure 1. Survival of *L. nigrinus* adults (maximum n per sample period is 12) and the average number of progeny found per sample period (n=54).

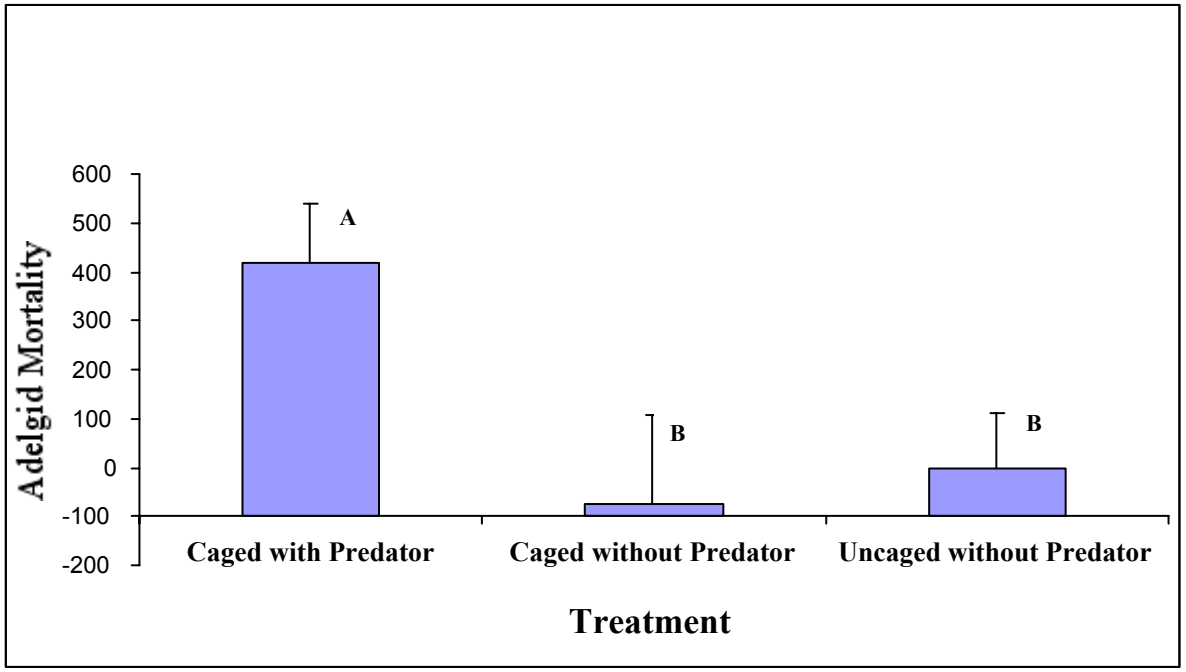

Figure 2. Mean mortality of adelgids in each treatment averaged across all sample periods in the field evaluation. Error bars indicate the standard deviation and different letters indicate a statistically significant difference between treatments (n=162).

2. Improving Rearing Techniques

Methods

Several experiments have been conducted to determine the critical factors necessary for successful rearing of *L. nigrinus*. The factors investigated during the 1st year of study were: duration of ovipositional period, larval density, soil type, moisture level and disturbance during pupation and aestivation. In addition, a new rearing cage was devised that enables us to count the number of larvae as they mature (Figure 3). The top section of the cage is an acetate cylinder with mesh screening on top. There is a coarse screen placed horizontally near the bottom of the cage, where floral foam with infested hemlock twigs and *L. nigrinus* eggs are placed. The bottom of the cage is a funnel attached to collecting jars. Infested hemlock branches are added weekly to allow larvae to feed and develop until they are ready to drop to the soil. Mature larvae are collected and counted as they migrate down to the soil in search of a pupation site.

Results

The new rearing cages allow us to quantify the number of larvae reaching maturity and estimate survival of pupae and aestivating adults. *L. nigrinus* egg production and larval success can be maximized by providing quality food (live adelgids) to the adults for a limited length of time (2-3 days) (Figure 4). Longer ovipositional periods did not yield a greater number of mature larvae ($F_{1,106}$= 0.53; p = 0.4689).

Egg and larval stages suffered 25% mortality when given a sufficient amount of quality food (~800 linear cm of infested hemlock branches at a density of 2 HWA/cm) (Figure 5). On average, *L. nigrinus* suffered 30% mortality during pupation at the moisture levels tested (Figure 6). Moisture level and disturbance of soil during summer aestivation may contribute to mortality of adults during this life stage, and will be assessed by adult emergence in October 2001.

Degree of success in rearing *L. nigrinus* from Oct. 2000 to Sept. 2001 will be fully assessed after adults emerge in fall 2001. Between February and June 2001, 7000 larvae were successfully reared to maturity from 350 adults (Table 1). To date (mid-Sept. 2001), over 800 adults have emerged from summer aestivation.

Investigations in the second year of the study will include testing higher densities of larvae per cage than those previously used, to determine the optimum number of mature larvae to be placed in each rearing cage. We will also examine how photoperiod, temperature, and soil type affect the survival of aestivating adults.

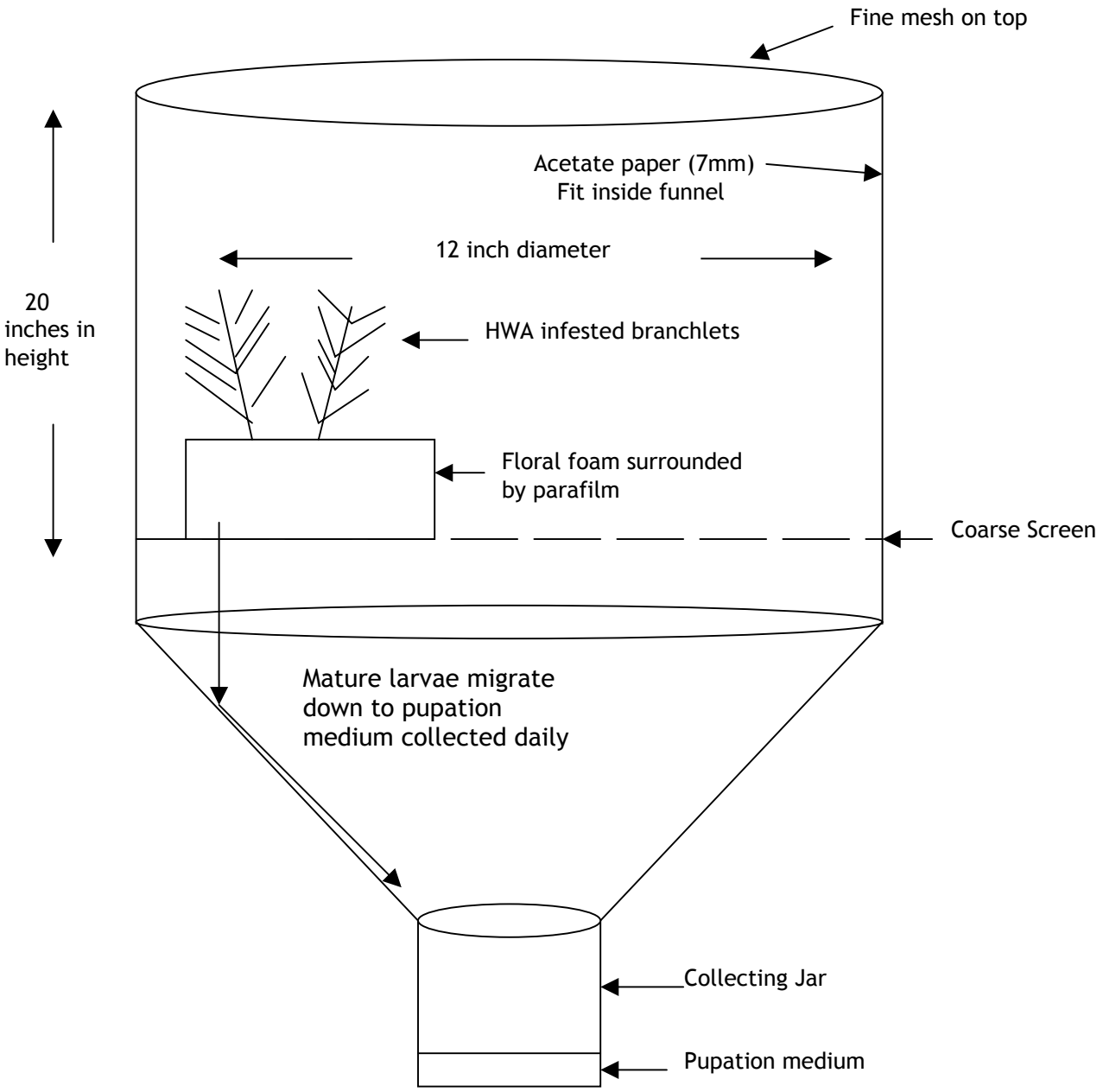

Figure 3. Modified cage design for rearing *L. nigrinus* eggs and larvae.

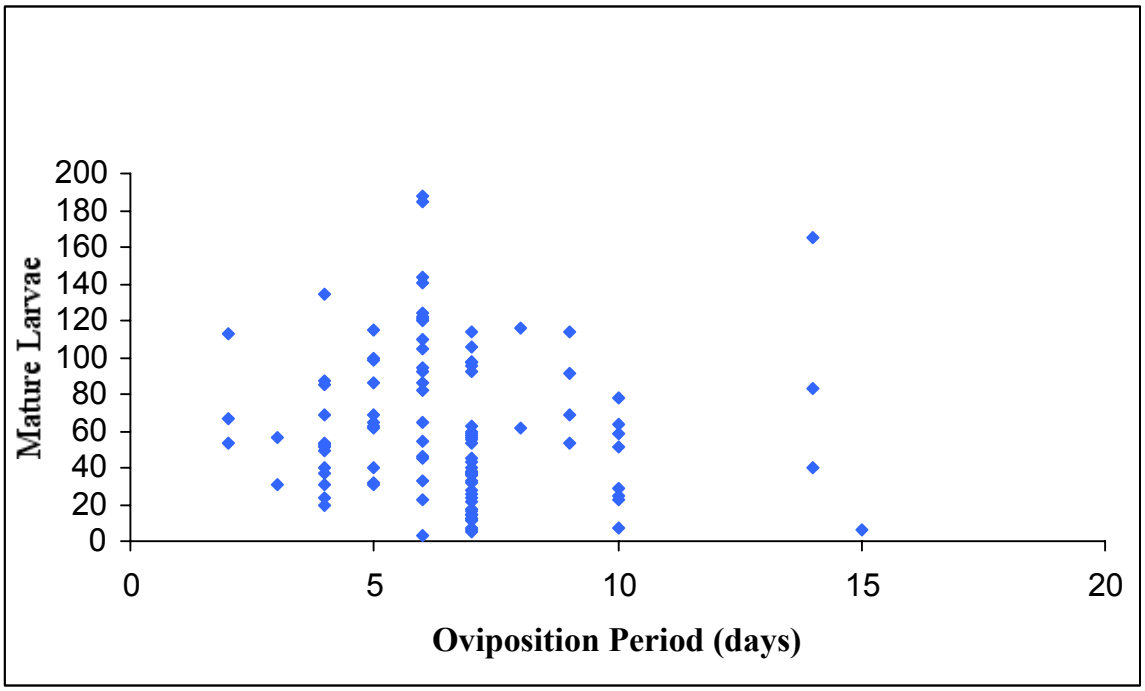

Figure 4. The observed relationship between the oviposition period and the number of larvae collected from each rearing cage.

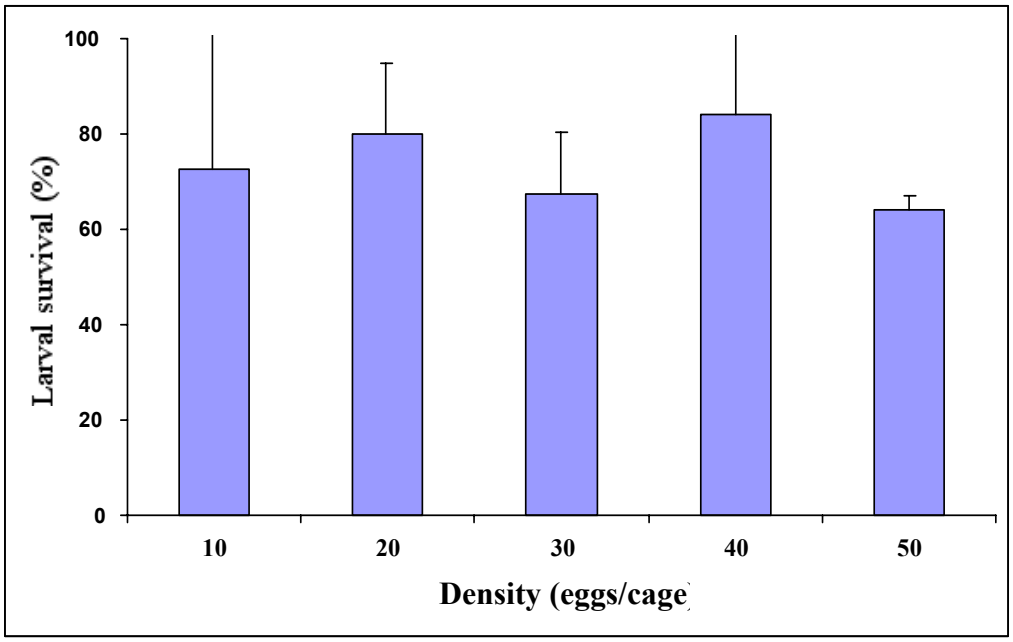

Figure 5. Mean survival of *L. nigrinus* larvae at various densities within a cage.

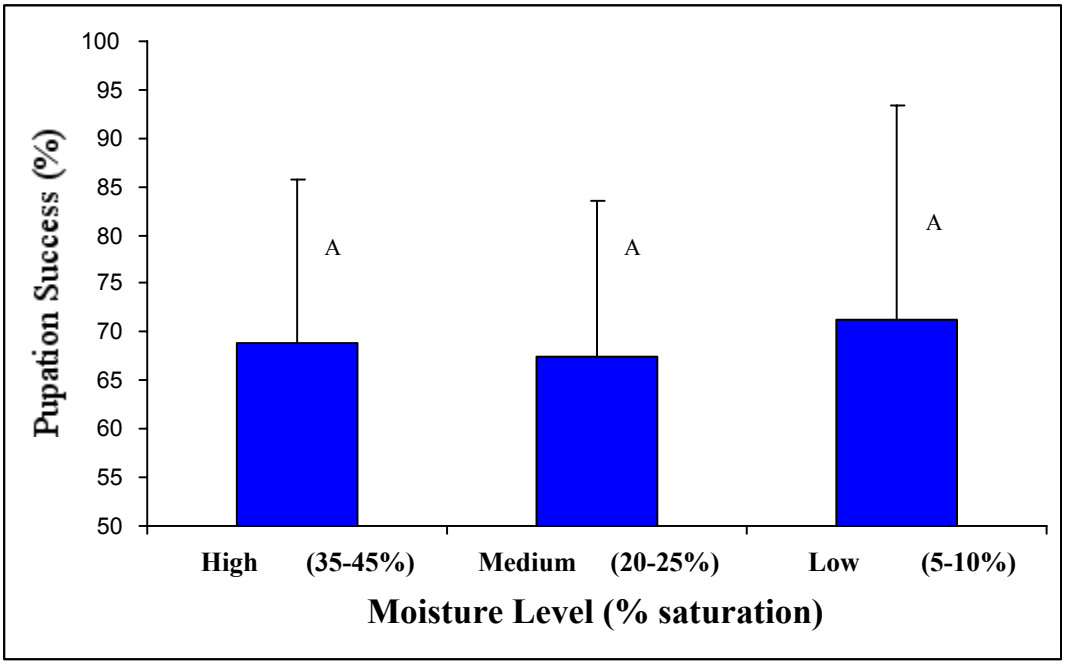

Figure 6. Mean percent of *L. nigrinus* that successfully completed pupation at different moisture levels. The error bars represent the standard deviation from the mean and similar letters indicate no significant difference between treatments (n=24).

Table 1. The number of individuals at each life stage of the *Laricobius nigrinus* colony between January and September 2001. Degree of success will be determined as adults break aestivation in October 2001.

***L. nigrinus* Life Stage**	**Estimated Success**	**Estimated Total**
F1 Adults	(collected in Jan. 2001)	350 reproductive adults
Larvae	~ 70%	~7000
Pupae	~ 69%	~5000
F2 Adults	(will be determined fall 2001)	

DOCUMENTATION:

Presentations Made:

Lamb, A.B., S.M. Salom, and L.T. Kok. *Laricobius nigrinus*, a predator of hemlock woolly adelgid, the next step in a biological control program. 10th Ann. So. For. Appal. Forest Entomol. and Pathol. Workshop. Crossnore, NC Feb. 15 - 16, 2001.

Lamb, A.B., S.M. Salom, and L.T. Kok. Progress in the evaluation of *Laricobius nigrinus* as a biological control agent against HWA. Southern Forest Insect Work Conference. July 23–26, 2001. Jekyll Island, Georgia.

FIRST YEAR FUNDED: FY 2001

YEAR SCHEDULED TO END: FY 2003

ACTUAL YEAR TO END: FY 2004

FIRST FISCAL YEAR FUNDED: FY2001----

FUNDS OBLIGATED FROM BEGINNING OF PROJECT THROUGH CURRENT FISCAL YEAR:
(include both monetary and in-kind, excluding FHP base funding and salaries) (extend table as needed)**:**

	Item	Requested Funding	Received Funding	Expended Funding
PREVIOUS YEAR FY 20001				
Administration	Salary	29,700	29,700	29,700
	Overhead			
	Travel	4,200	4,200	4,200
Procurements	Contracting	1,000	1,000	1,000
	Equipment			
	Supplies	5,000	5,000	5,000
YEAR TOTALS		39,900	39,900	39,900

CURRENT YEAR FY 2002				
Administration	Salary	30,100		
	Overhead			
	Travel	4,600		
Procurements	Contracting	1,000		
	Equipment			
	Supplies	5,000		
YEAR TOTALS		40,700		

Enclosure 3 continued

FY 2003		Requested FHP STDP Funding	Other Source Funding	Source
Administration	Salary	31,500	39,475	VA Tech
	Overhead			
	Travel	4,600		
Procurements	Contracting			
	Equipment			
	Supplies	5,400		
YEAR TOTALS		41,500		
PROJECT TOTALS		122,100	116,145	

FUNDS NOT USED FROM PREVIOUS FISCAL YEAR (If there are unused funds, what is the reason for not using them? How will the project continue without these funds?)

Fiscal Year	STDP Funding Allocated	Funds Obligated	Funds Unused
All allocated funds used			

EXPECTED BUDGET FOR NEXT FISCAL YEAR: (include both monetary and in-kind, excluding FHP base funding and salaries) (extend table as needed)**:**

	Item	Requested FHP STDP Funding	Other-Source Funding	Source
Administration	Salary	30,100	38,715	
	Overhead			
	Travel	4,600		
Procurements	Contracting	1,000		
	Equipment			
	Supplies	5,000		
Totals		40,700	38,715	VA Tech

DIFFERENCE BETWEEN ORIGINAL AND AMENDED REQUESTS AND JUSTIFICATION (the difference between originally requested funds and funds needed based on changes in the budget or scope of the project. Be specific and clear about where the money will be used and by whom)**:**

STDP FUNDING NEEDED:

Total estimated additional future funding needed beyond the current fiscal year: 41,500

Estimated STDP funding needed in remaining year(s) of the project by fiscal year. Show separately the funding to be requested/provided from other sources (extend the table as necessary).

Fiscal Year	STDP Funding	Other-Source Funding	Source
FY 2003	41,500	39,475	VA Tech