ANALYSIS OF MICONIA DATA

STATISTICAL REPORT

Prepared for

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October 2000

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II. EXECUTIVE SUMMARY

This report consists of a field release protocol and a field sampling protocol for the phytopathogenic fungus *Colletotrichum gloeosporioides* f. sp. *miconiae* as a biological control agent for *Miconia calvescens*. The protocols were used by Hawaii State Department of Agriculture employees and their collaborators to monitor the post release impact of the fungus on *M. calvescens* vigor as measured by height growth and leaf drop, and the effects of four application additives on the establishment of the fungus on *M. calvescens*. The protocols were designed to be statistically sound and to provide a basis for analysis of the data collected that yielded inferences about the establishment of the fungus, its impact on *M. calvescens* vigor, and the effects of application additives.

The field release protocol identifies at each site, those plots that will be treatment plots and those plots that will be control plots. The release protocol also provides information about field procedures for the application of the fungus on *M. calvescens* in each of the treatment plots, including; 1) the selection of trees on which the 4 application additives will be used; 2) where to place flags identifying leaves present before application of the fungus; and 3) environmental characteristics to be measured at time of fungus release.

The field sampling protocol describes in detail the sampling procedure, including 1) how to select *M. calvescens* leaves on which fungus-caused lesions will be enumerated; 2) how to quantify leaf drop; and 3) a sampling schedule.

This report also includes the results of statistical analysis of *M. calvescens* leaf-drop data collected in 1999. The data were received from the on October 28, 1999. After consultation with Pat Conant and Eloise Kilgore, analysis of the data began October 23, 2000. Questions of interest were defined after an initial consultation and discussion. A list of those questions of interest is included in this report.

The report includes a discussion of the structure of the data, explanatory and response variables, and the statistical procedures employed in the analysis. The results of the analyses are reported along with inferences and p-values.

Poisson regression in a generalized linear regression format was used to analyze the count data. There was no evidence of extra dispersion in the model. (p-value = 0.9815). There was no evidence of a treatment by additive interaction (p-value = 0.2130). There was inconclusive evidence of an application additive effect (p-value = 0.1212). Median leaf-drop was 19.3% higher for Alginate Solution than for water. Median leaf-drop was 19.1% higher for Silwel L-77 than for water. Median leaf-drop was 15.4% higher for Sucrose Solution than for water. Median leaf-drop was 10.8& higher for Kaolin than for water. There was strong evidence of a treatment effect (p-value < 0.0001). Plants exposed to the fungus dropped 22.8% more leaves than those not exposed to the fungus.

III. INTRODUCTION

Over 4,600 plant species have reached the Hawaiian island chain over the last 200 years and over 600 of these species have established populations in Hawaii (Smith 1985). Several of these species pose threats to Hawaii's unique ecosystems and to the sustainable use of agricultural lands. Eighty-five alien plant species have been identified as having a disruptive impact on the natural processes of native ecosystems and are considered to be serious pests (Smith 1985).

Miconia calvescens is a species of the Melastomataceae family, all members of which are considered pest species in Hawaii. The family contains invasive species like *Clidemia herta* and *Tobouchina urvilleana*, which have been declared noxious weeds in Hawaii, and other threatening species, including *M. calvescens*. A review of *M. calvescens*, its introduction, and its potential for environmental damage in Hawaii can be found in the Environmental Assessment for release of the phytopathogenic fungus *Colletotrichum gloeosporioides* f. sp. *miconiae* as a biological control agent (Kilgore et al. 1997).

The fungal pathogen, *C. g. miconiae*, was isolated from lesions on *M. calvescens* leaves in Brazil (Kilgore et al. 1997). Based on its ability to cause leaf spotting and its potential to cause leaf-drop on *M. calvescens*, *C. g. miconiae*, was considered a potentially useful biological control agent. The fungus was tested for its effects on closely related melastome species, two genera (*Metrosideros* and *Wilstroemia*) of Hawaiian endemic plants, one Hawaiian indigenous plant species (*Eugenia reinwardtiana*), several landscape and ornamental plants and cultivated forest trees. Of all the plants tested, *Miconia calvescens* was the only susceptible host (Kilgore et al. 1997). The release of *Colletotrichum gloeosporioides* f. sp. *miconiae* was "expected to have no adverse effects either on threatened and endangered plant species or human and animal health in Hawaii" (Kilgore et al. 1997).

Based on laboratory observations, *C. g. miconiae* should cause premature leaf drop. Other effects are unknown, but *M. calvescens* panicles may become infected and fruiting could be reduced. No effort will be made to quantify this effect, although field notes may provide information for future studies.

The Hawaii State Department of Agriculture has obtained a grant from the U.S. Forest Service to release this fungus in Hawaii and evaluate its potential control of *M. calvescens*.

Biological control of weeds is a science that has evolved in the Twentieth century. The first attempt at controlling weeds with insects occurred in Hawaii in 1902 and was rapidly adapted for use on the mainland and around the world. First attempts at biological control of weeds were aimed at plants causing some sort of economic damage to cropping systems. In the past ten years greater attention has been given to study of biological control of weeds in natural ecosystems.

One of the main dangers weed control scientists must be aware of is damage that released biological control agent may cause in native ecosystems. Standardized protocols have been developed for the testing under quarantine of candidate biological control agents and their subsequent release. However, these protocols give little guidance for the evaluation of the effectiveness of the control and its cost benefits. The success of biological control programs requires scientific feedback to determine the effectiveness of the method and to provide options for improving the control.

Biological control methodology is not benign. Care should be taken to evaluate not only the effectiveness of the released control agents on the target species but also to determine the effect of biological control agents on desirable, indigenous species. Funding is not provided in this project for these valuable evaluations critical for environmental safety.

The State of Hawaii, Department of Agriculture sampled the study sites during 1999.

IV. PROJECT OBJECTIVES

1. To develop effective means of delivering and establishing *Colletotrichum gloeosporioides* f. sp. *miconiae* as a biological control agent on *Miconia calvescens*.

2. To develop and implement methodology for post-release evaluation of the effects of *Colletotrichum gloeosporioides* f. sp. *miconiae* on *Miconia calvescens* vigor as measured by height-growth and leaf-drop.

3. To test the effectiveness of *Colletotrichum gloeosporioides* f. sp. *miconiae* to cause leaf-drop in *Miconia calvescens*.

4. To test the synergism of 5 spray additives with *Colletotrichum gloeosporioides* f. sp. *miconiae* to cause leaf-drop in *Miconia calvescens*.

Summary of the experiment and objectives

This study is an experiment designed to evaluate the effects of *Colletotrichum gloeosporioides* f. sp. *miconiae* as a biological control agent for *Miconia calvescens.* Five application additives were tested to evaluate their synergism with the fungus and its ability to cause leaf-drop in *Miconia calvescens*.

Questions of Interest

1. Does the fungus *Colletotrichum gloeosporioides* f. sp. *miconiae* cause premature leaf-drop in *Miconia calvescens*?

2. Is there a difference in leaf-drop in *Miconia calvescens* between 5 application additives?

Populations of Interest

The population of interest is the number of leaves that drop from tagged stems of *Miconia calvescens*.

Structure of the Experiment

Experimental Units

Individual *Miconia calvescens* trees.

Response variable

Number of leaves that drop from tagged stems of *Miconia calvescens*.

Explanatory variables

Four sites where paired plots of *Miconia calvescens* were established.

- Site 1, Waiakea uka mauka plot and Waiakea uka makai plot.
- Site 2, Suefuji drainage plot and UH taro patch plot.
- Site 3, Palm tree plot and banana patch plot.
- Site 4, Suefuji Railroad plot and Boyd's Railroad plot.

Three size classes of *Miconia calvescens.*

- Class 1, small
- Class 2, medium
- Class 3, large

Five application additives

- Alginate Solution
- Sucrose Solution
- Silwel L-77
- Kaolin
- Water

Two Treatments

T1 = Spray with *Colletotrichum gloeosporioides* f. sp. m*iconiae*
T2 = Spray with no fungus

Analysis Of Variance Table

Source Of Variation	Degrees of Freedom
Site	3
Size class	2
Application additive	4
Treatment	1

V. FUNGUS FIELD RELEASE PROTOCOL

Four sites, each containing two plots have been established by Pat Conant on the east side of the island of Hawaii. Sites have been paired for similar environmental conditions. The sites and paired plots are as follows. Site 1, Waiakea uka mauka plot and Waiakea uka makai plot. Site 2, Suefuji drainage plot and UH taro patch plot. Site 3, Palm tree plot and banana patch plot. Site 4, Suefuji Railroad plot and Boyd's Railroad plot. Treatment plots have been randomly assigned as Site 1, Waiakea uka mauka plot, Site 2, Suefuji drainage plot, Site 3, Palm tree plot, and Site 4, Suefuji Railroad plot. Remaining plots will be control plots. Rain gauges will be installed at each plot.

Within each of the plots, Miconia calvescens trees have been selected by Pat Conant in three size classes for inclusion in the study. Trees were selected for their accessibility and distance from other selected trees.

Plastic flagging will be tied to each of the trees to be included in the study just below the top pair of fully opened leaves. The number of leaves below the flagging will be recorded.

If possible, the release of the fungus will occur on the same day for all plots. On this day, as the fungus is released on each plot, the field leader will record, time of day at start of release and at end of release; relative humidity; air temperature, level of water in the rain gauge on that plot and the height of each tree included in the study.

On the treatment plots, inoculation spore concentrations of 1 x 106 spores per ml in water will be applied with each application additive. Separate spray applicators will be used for each of the application additives to avoid contamination. On the control plots, trees will be sprayed with the application additives but with no inoculation spores.

An incubation period of two months will be allowed for establishment of the fungus before field sampling begins.

VI. FIELD SAMPLING PROTOCOL

Hypotheses:

The establishment of the fungus, Colletotrichum gloeosporioides f. sp. miconiae on Miconia calvescens as measured by the number of lesions per leaf will be affected by application additives, kaolin, sucrose solution alginate solution, and surfactant.

The fungus, Colletotrichum gloeosporioides f. sp. miconiae, will affect Miconia calvescens vigor as measured by tree height-growth and the number of leaves dropped.

Sampling Protocol:

After a two month incubation period, field sampling will begin. The number of lesions on the lowest two leaves will be counted bi-monthly on each of the trees included in the study.

Leaves on Miconia calvescens are paired and if one of the pair of the lower whorl has dropped, only the lesions on the lowest single leaf need be counted. In other words, every two months, the lesions on the lowest one or two leaves will be counted on each tree included in the study. This includes trees on control plots as well as treatment plots. Field personnel will record date of sample, name of plot, number of tree, height of tree, the number of leaves remaining below the flagging, number of leaves on which lesions were counted, and the number of lesions counted. Data will be entered in field notebooks and entered into a spreadsheet.

VII. STATISTICAL PROCEDURES

Poisson Regression for Count data

The Poisson probability distribution is useful for describing the population distribution of counts of occurrences of some event over time or space. The distribution has been used in a wide variety of situations and is most appropriate for counts of rare events that occur at random points in space or time. The number of leaves that drop form a stem is example of these types of data.

The probability of obtaining Y successes is given by the formula

$$\text{Probability}\{Y\} = \exp(-\mu)*\mu^{Y}/Y!, \text{ for any } Y = 0,1,2, \ldots$$

The features of the Poisson distribution allow regression models to account for increasing variance with increasing means response. In the Poisson distribution, the variance is equal to the mean.

Poisson log-linear regression models specify that the logarithms of means of Poisson responses are linear in regression coefficients. Coefficients of the Poisson regression model can be estimated using a generalized linear model. The generalized linear model with a Poisson response is

$$\mathit{log}\,(Y) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + . .$$

Where μ = the mean of the Poisson response, β_0 is the intercept coefficient, β_1 is the coefficient for the first explanatory variable, X_1, and β_2 is the coefficient for the next explanatory variable, X_2.

Many times analysis of Poisson distributed data is conducted with transformed data. The logarithm of the response often straightens out the relationship between the response and the explanatory variables. The variance remains non-constant and normal regression analysis often fails to arrive at a parsimonious model. The transformation that stabilizes the variance is the square root of the response, but interpretation is not satisfactory. The Poisson log-linear approach does not require a transformation and is the most suitable approach to modeling count data.

The maximum likelihood method is used to estimate the coefficients in Poisson log-linear regression. The parameters for the model are those that yield the highest probability of observing precisely what was observed. The method is calculation intensive and is used in most modern statistical software packages. A thorough discussion of Generalized Linear Regression and Maximum Likelihood Estimation of model parameters is contained in *Generalized Linear Models 2nd Edition* (McCullagh and Nelder 1991)

Unmeasured effects, clustering of events, or other factors sometimes produce more variation in the responses than is predicted by the Poisson model. Using Poisson log-linear regression when extra-Poisson variation is present yields roughly unbiased estimates of parameters, but standard errors are smaller than reality and tests of significance yield smaller p-values than are truly warranted by the data. A quasi-likelihood approach can be applied to extend the model when extra-Poisson variation is present. The quasi-likelihood approach accounts for the larger than expected variation and gives tests and resulting p-values adjusted for the extra-Poisson variation. The extra-Poisson variation is summarized in the dispersion parameter.

Software used for Poisson log-linear regression was S-Plus version 4.5 (MathSoft 1988-1998).

VIII. SUMMARY STATISTICS

For each site, the mean and standard error of the number of leafs dropped from tagged stems of *Miconia calvescens* are given in Table 1.

SITE	MEAN	STD. ERR.
Site: a	6.46	0.20
Site: b	5.84	0.21
Site: c	4.99	0.26
Site: d	6.01	0.27

Table 1. Site Summary Statistics.

For each size class, the mean and standard error of the number of leafs dropped from tagged stems of *Miconia calvescens* are given in Table 2.

SIZE CLASS	MEAN	STD. ERR.
Small	5.68	0.16
Medium	5.76	0.21
Large	6.22	0.34

Table 2. Size Class Summary Statistics.

For each treatment, the mean and standard error of the number of leafs dropped from tagged stems of *Miconia calvescens* are given in Table 3.

TREATMENT	MEAN	STD. ERR.
No Fungus	4.88	0.16
Fungus	6.63	0.16

Table 3. Treatment Summary Statistics.

For each application additive, the mean and standard error of the number of leafs dropped from tagged stems of *Miconia calvescens* are given in Table 4.

ADDITIVE	MEAN	STD. ERR.
Alginate	6.55	0.28
Kaolin	5.99	0.24
Silwel L-77	6.43	0.27
Sucrose	6.17	0.35
Water	4.66	0.19

Table 4. Application Additive Summary Statistics.

IX. SUMMARY OF LEAF DROP POISSON REGRESSION ANALYSIS

Poisson Regression Analysis

Parameters were estimated for a Poisson log-linear model of changes in the response of leaf drop explained by site, size class, application additive, and treatment. The Poisson log-linear regression model was

$$\log(Y) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \ldots$$

Where Y is the mean of the response variable (number of dropped leaves), X_1, X_2, X_3 are the explanatory variables, β_0 the coefficient for the intercept, β_1 the coefficient for the first explanatory variable, X_1, β_2 the coefficient for the next explanatory variable, X_2,. etc.

The summary statistics of the estimated coefficients of the final model are in Table 5.

Inference:

There is no evidence of extra dispersion in the model. (p-value = 0.9815).

There is no evidence of a treatment by additive interaction (p-value = 0.2130).

There is inconclusive evidence of an application additive effect (p-value = 0.1212). Median leaf-drop was 19.3% higher for Alginate Solution than for water. Median leaf-drop was 19.1% higher for Silwel L-77 than for water. Median leaf-drop was 15.4% higher for Sucrose Solution than for water. Median leaf-drop was 10.8& higher for Kaolin than for water.

There is strong evidence of a treatment effect (p-value < 0.0001). Plants exposed to the fungus dropped 22.8% more leaves than those not exposed to the fungus.

There is no evidence of a lack of fit of the final model to the data (p-value = 0.5671).

Coefficients		Value	Standard Error	t-statistic
Intercept =	β_0	1.906221428	0.09297250	20.50306744
Site b =	β_1	-0.106060873	0.05813176	-1.82449108
Site c =	β_2	-0.267928390	0.06613350	-4.05132620
Site d =	β_3	-0.129906661	0.06620872	-1.96207771
Size class Small =	β_5	-0.097663514	0.05817037	-1.67892204
Size class Medium =	β_4	-0.097238592	0.06009694	-1.61802911
Alginate =	β_6	0.17663482	0.08023472	2.201476
Silwel L-77 =	β_7	0.17476761	0.07933354	2.202947
Sucrose =	β_8	0.14316320	0.08082883	1.771190
Kaolin =	β_9	0.10243767	0.08271008	1.238515
Fungus =	β_{10}	0.205791315	0.05925426	3.47302159

Dispersion Parameter for Quasi-likelihood family taken to be 2.530334
Null Deviance = 407.1451 on 395 degrees of freedom
Residual Deviance = 330.4488 on 385 degrees of freedom

Table 5. Estimated Poisson Generalized Linear Regression final model parameters and summary statistics of leaf-drop count data.

Bench Notes that provide details of the analytical procedures, S-Plus commands, and statistical output of the Poisson Generalized Linear Regression models are available upon request.

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