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NITROGEN FIXING TREE ASSOCIATION
P. O. Box 680
Waimanalo, Hawaii
96795, U.S.A.

SUMMARY OF ACCOMPLISHMENTS

1. TITLE:

INTERNATIONAL *LEUCAENA* TRIALS FOR PSYLLID RESISTANCE
Grant No. DHR-1406-G-SS-6051-00

2. PRINCIPLE INVESIGATORS:

J.L. Brewbaker, Nancy Glover and Robert Wheeler

3. SUBMITTING INSTITUTION:

Nitrogen Fixing Tree Association (NFTA)
P.O. Box 680
Waimanalo, Hawaii 96795 USA

4. OBJECTIVES:

1. Establish policies and strategies for collaborative research and development on the genetic and biological control of the *Leucaena* psyllid (*Heteropsylla cubana*).

2. Establish a network of international collaborators working with *Leucaena* species.

3. Develop standardized procedures for collaborative research on genetic control of the *Leucaena* psyllid.

4. Evaluate *Leucaena* germplasm for psyllid resistance through the international network.

5. Multiply *Leucaena* germplasm of proven value for psyllid resistance.

5. ACCOMPLISHMENTS:

5-1. **Summary**: Five goals were set for the establishment of definitive international provenance trials of *Leucaena* spp. for resistance to a serious insect pest, the psyllid *Heteropsylla cubana*. Leucaena is estimated to be grown economically on over 2 million hectares, and loss to the psyllid was great during its first year of attack. The grant of $50,000 was disseminated largely for the conduct of an international workshop to define genetic and biological control strategies, and for a series of standardized resistance trials in 7 countries. Modest fiscal support was provided for each of the 12 collaborating research stations, of which 11 were able to complete the trials as planned. Outstanding resistance was identified with regional stability. Seed production has been initiated under a separate grant from Winrock. These limited trials illustrate the need for an international collaborative network for evaluation of leucaena germplasm and management strategies.

Phone: (808) 259-8555 Cable Address: NFTAHAWAII, Hawaii Telex: 510100 4385

5-2. Objective 1: A workshop on the Biological and Genetic Control Strategies for the *Leucaena* Psyllid was held in Hawaii, November 3-7, 1986 and attended by 47 participants. Ten invitational papers were presented on the biology and natural enemies of the psyllid, and genetic control options using tolerant and/or resistant *Leucaena* species and hybrids. Each country represented at the workshop reported psyllid damage and their psyllid control programs. Participants divided into four working groups -- *Leucaena* Improvement, Network Trials, Psyllid Biology and Natural Enemies, and Technology Transfer and Policy Issues-- to produce an Action Plan. The proceedings were published as a special issue of the annual NFTA publication, THE LEUCAENA RESEARCH REPORTS. Copies were made available gratis to participants and NFTA associates and at cost to others.

5-3. Objective 2: An international network of collaborators was established in 1986, primarily in regions where *Leucaena* is an important tree crop and presently under psyllid pressure (Table 1). In 1987, seven new collaborators were added to the network in countries where the psyllid has recently arrived (Table 2).

Table 1. Collaborators in the 1987 Network.

Trial No.	Collaborator	Location	Date Planted
LPT 87-01	Bob Wheeler, NFTA	Hawaii, USA	6/87
LPT 87-02	Jose Delgado, INIREB	Mexico	7/87
LPT 87-03	L. Wilson, Univ. of WI	Trinidad	--
LPT 87-04/05	Enrique Crizaldo, ERDB	Philippines (Nueva Ecija)	6/87
LPT 87-06	J. Luego, Forestry Bureau	Philippines (Cebu)	8/87
LPT 87-07	R. Escalada, ViSCA	Philippines (Leyte)	3/87
LPT 87-08	K. Yantasath, TISTR	Thailand	7/87
LPT 87-09	C. Piggins	Indonesia	5/87
LPT 87-10/11	I. Oka	Indonesia	11/87
LPT 87-12	Bob Bray, CSIRO	Australia	11/87

Table 2. Collaborators in the 1988 Network

Trial No.	Collaborator	Location
LPT 88-01	M.J. Williams, USDA	Florida, USA
LPT 88-02	K. Krishnamurthy, Univ. of Ag.	India
LPT 88-03	P. Robinson, Dept. of Forestry	Nepal
LPT 88-04	Narayan Hegde, BAIF	India
LPT 88-05	C.K. Ong, ICRISAT	India
LPT 88-06	K. Vevekanandan, Forest Dept.	Sri Lanka
LPT 88-07	H.P.M. Gunasena, Univ. of Peradeniya	Sri Lanka

5-4. Objective 3: Standardized procedures for the trials were developed at and following the workshop to include nursery management, trial design, trial management, data collection procedures and data analysis (Appendix 1). A collaborative agreement with the British Museum was set up for the identification of predators collected by all collaborators in the network.

The trials were specifically designed for regular fodder harvest. The degree of psyllid infestation (eggs, nymphs and adults), psyllid damage, and predator counts are being measured monthly. Growth measurements are taken at 6 months and at 3 month intervals thereafter.

5-5. Objective 4: Seeds, *Rhizobium* inoculum and standardized guidelines were provided to all collaborators. *Leucaena* species and hybrids evaluated in the trials are given in Table 3.

Table 3. *Leucaena* species and hybrids evaluated in the Network.

K No.	Species
K500	L. leucocephala (Cunningham)
K8	L. leucocephala (Hawaiian Giant)
K636	L. leucocephala
K527	L. leucocephala
K614	L. leucocephala
Local	L. leucocephala
K156	L. diversifolia 4N
K784	L. diversifolia 4N
K785	L. diversifolia 4N
CSIRO #46568	L. diversifolia 2N
CSIRO #33820	L. diversifolia 2N
K376	L. pallida
CFI 45/85	L. collinsii
KX1	L. diversifolia x L. pallida
KX2	L. leucocephala x L. pallida
KX3 (K743)	L. diversifolia x L. leucocephala

Preliminary results from the trials include the outstanding psyllid resistance and fast-growth of the hybrids KX1 and KX2, *L. pallida*, and the two varieties of *L. diversifolia* K784 and K785 (Figure 1 and Table 4). *L. leucocephala* K636 outperformed the other *L. leucocephala* varieties which appears to be a result of its ability to recover much faster from psyllid damage. Interestingly, under low psyllid infestation (LPT 87-07), the giant *L. leucocephala* varieties, the most widely planted *L. diversifolia* (K156) and the hybrid KX3 outperformed the other entries.

Figure 1. Psyllid damage (average rating of 7 months) in Hawaii.

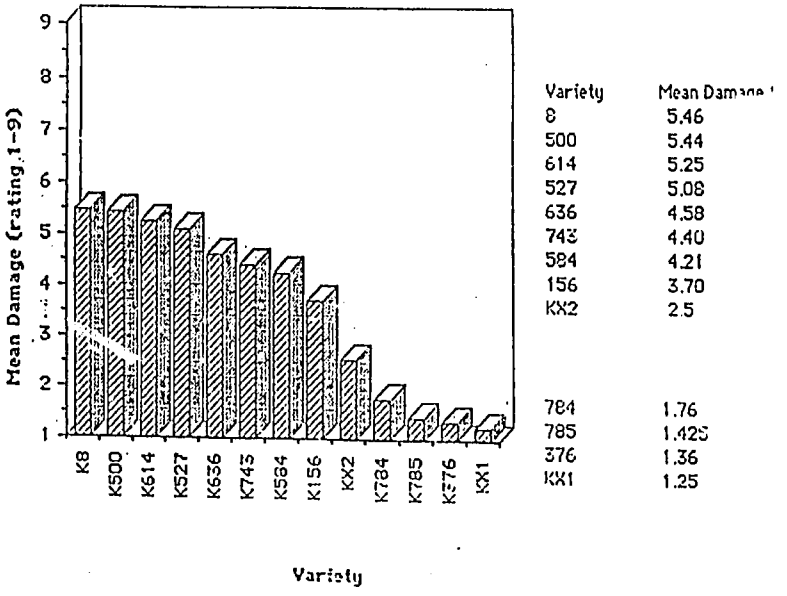


Table 4. Height growth at 6 months of age, before cutting.

K No.	LPT 87-01	LPT 87-06	LPT 87-07 *
	- m -	- m -	- m -
KX1	3.2	2.8	3.4
K376	2.9	2.2	3.0
K785	2.7	2.5	2.7
KX2	2.6	2.4	3.0
K784	2.6	1.9	3.0
K636	1.9	1.6	3.8
K8	1.8	0.7	3.4
K156	1.7	1.4	3.5
K584	1.7	---	---
K614	1.7	---	3.4
K500	1.7	1.1	2.9
KX3	1.5	1.0	3.9
K527	1.3	0.7	3.5
Local	---	0.4	2.9

* Height at 9 months of age

The Network has provided us with the opportunity to verify the methodology used to evaluate psyllid resistance. Psyllid damage ratings (empirical, 1-9 scale) best reflect biomass increments under infestation. Ratings based on numbers of psyllid nymphs are well correlated to damage, although adult and egg values are not. This methodology has proven to be easy-to-use and not time consuming.

The collaborators in the LPT Network have continued taking measurements in their trials beyond the grant date (March 1988). A scholarly scientific paper is in preparation to include all trial data and will be published in the 1988 LEUCAENA RESEARCH REPORTS.

5-6. Objective 5: In collaboration with F/FRED, NFTA is coordinating a series of seed production areas in Asia for selection and multiplication of resistant varieties and hybrids. Guidelines are in preparation and seed will be distributed so that each seed production area will have a specific plant breeding/seed increase responsibility (eg. fodder and green manure use in seasonally dry tropical lowlands, fuelwood use in sub-tropical midlands, etc.). Seed will be produced in collaboration with BAIF, India; TISTR, Thailand; ViSCA, Philippines; AARD, Indonesia and FRI, Taiwan.

6. RESEARCH NEEDS:

The identification of psyllid resistant *Leucaena* germplasm and the development of resistant hybrids is a major advance in the control of this pest. Only through network trials can regional stability of such resistance be confirmed. There is need for continuing evaluations of other hybrids which have outstanding potential which include crosses with the most tolerant variety of *L. leucocephala* (K636) and the resistant varieties of *L. diversifolia* (K784, K785). Crosses with the *Leucaena* species immune to the psyllid (*L. pallida*,

L. collinsii, *L. retusa*, *L. salvadorensis*, *L. esculenta*) should be developed for evaluation and ecological adaptability.

New leucaena germplasm is rapidly accruing internationally, and an international network of fodder and wood production trials is needed. Such trials could undergird the broadening of germplasm base, to prevent in the future events such as the rapid and damaging dispersal of the psyllid. Many species, hybrids, and provenances promise improved quality, improved yield, and new uses for leucaenas in tropical agroforestry.

Vegetative propagation and grafting techniques need to be refined to permit hybrid seed production based on self-incompatibility, and to allow plantation development with seedless trees. Fodder quality evaluations of psyllid resistant *Leucaena* germplasm are also needed.

GUIDELINES FOR LEUCAENA PSYLLID TRIALS (LPT)

The following guidelines describe standardized procedures for LPT management, da collection, and progress reports.

All LP Trials should be managed in a standardized way in order to obtain relevant data for analysis and interpretation (i.e. environment x psyllid infestation, genetic resistant stability, etc.).

NURSERY MANAGEMENT

Leucaena seeds will not germinate well unless they are scarified. Two methods ar used satisfactorily and are described below.

1. Scratch or nick the round end of each seed with a knife, small scissors, fingernail clipper or file.
2. Pour boiling water over seeds (5-10 times as much volume of water as seed), stir gently, pour off in three minutes, replace with tap water and soak overnight.

No pre-treatment is necessary for the Gliricidia sepium seed.

Rhizobium inoculation is necessary for good nodulation and growth. A slurry should be made of the inoculum using a sticker such as gum arabic. Seeds are immersed in the slurry to be coated with inoculum. Seeds can be air dried in a cool place to make planting easier or planted immediately. Inoculated seed must be planted shortly after drying or the *Rhizobium* will be killed by lack of moisture.

TRIAL DESIGN

The trial is a randomized complete block design (see attachment) with the following characteristics:

3 replications
12 entries
2 row plots, 5 meters long
1 meter between rows, .25 m between plants within rows (40 trees/plot), and 2 meters between plots

Entries:

L. leucocephala K636
L. pallida K376
L. collinsii
Hybrid KX1 (L. diversifolia x L. pallida)
Hybrid KX2 (L. leucocephala x L. pallida)
Hybrid KX3 (L. diversifolia x L. leucocephala)
L. diversifolia K156
L. diversifolia #46568
L. diversifolia #33820
L. leucocephala K8
Gliricidia sepium
L. diversifolia K785

If germination is low for some entries and there are not enough seedlings for 3 replications, one or two replicates can be used.

CUTTING SCHEDULE

The trial is to be managed as hedge lines for fodder production. Trees are to be cut back to 50 cm above ground level after 6 months of growth or when the average height of the trial reaches 1 meter. Subsequent cuttings should be cut to 75 cm above ground level every 3 months or when the average height of the trial reaches 1 meter. All entries must be cut at the same time.

MEASUREMENTS

1. PSYLLID COUNTS AND PSYLLID DAMAGE.

Two types of data are to be taken on the psyllid--counts and damage.

1.1 Pyllid counts are to be scored empirically (1-9 rating) on a monthly basis. A large, hand-held magnifying glass (8 cm in diameter) can be used to count psyllids in all three stages (adults, nymphs and eggs). The scoring is to be done in the early morning on juvenile leaves at the end of growing stems. Seven cm of a juvenile leaf comprises one sample (Figure 1). **The sample must be competely foliated.** Juvenile leaves with opened leaflets are prefered samples, but scoring can be done on unopened leaflets if necessary. Eight trees are to be scored for each entry in each replication.

When psyllid infestation is abundant, it may be difficult to find standardized samples due to defoliation. If no samples can be found, a rating of 10 should be used for adults, nymphs and eggs.

<u>Psyllid Count Rating Scale</u>:

Adults	Nymphs	Eggs (% leaf cover)
1- No adults	1- No nymphs	1- No eggs present
2- 1-5 adults	2- 1-5	2- Occassional eggs present
3- 6-10	3- 6-10	3- Eggs (not masses) on most leaflets
4- 11-15	4- 11-15	4- Egg masses on $\leq$ 10% of leaflets
5- 16-20	5- 16-20	5- Egg masses on 25% of leaflets
6- 21-30	6- 21-30	6- Egg masses on 50% of leaflets
7- 31-40	7- 31-40	7- Egg masses on 75% of leaflets
8- 41-50	8- 41-50	8- Egg masses cover leaflets
9- 50+	9- 50+	9- Egg masses cover leaves and stems
10-Total defoliation	10-Total defoliation	10- Total defoliation

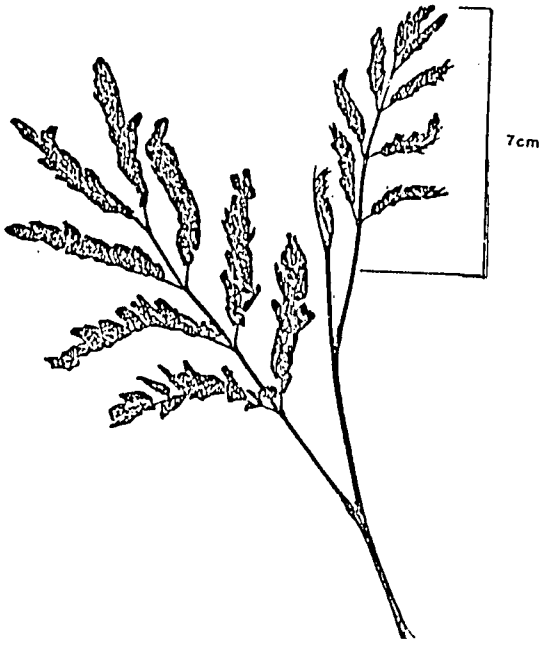


Figure 1. Sample area for psyllid counts.

1.2 Psyllid damage is to be scored empirically (1-9 rating) on entire terminal shoots (Figure 2). These shoots must be different than those used for psyllid counts, since psyllid counts must be done on selected, completely foliated samples. Eight trees are to be scored for each entry in each replication.

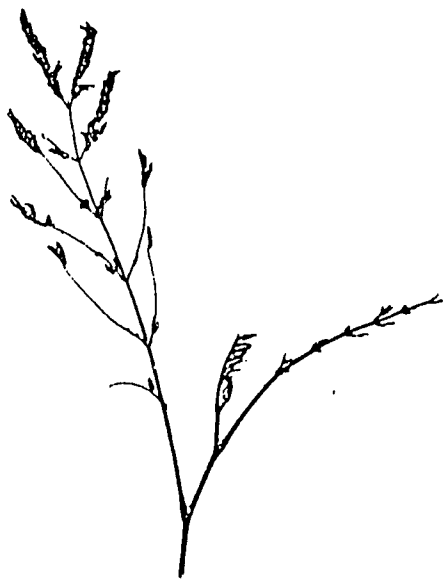
<u>Psyllid Damage Rating Scale:</u>

1- No damage observed
2- Slight curling of leaves
3- Tips and leaves curling and yellowish
4- Tips and leaves badly curled and yellowish
5- Loss of up to 25% of young leaves
6- Loss of 26 to 50% of young leaves
7- Loss of 51 to 75% of young leaves
8- 100 % loss of leaves and blackening of lower leaves
9- Blackened stem with total leaf loss

Figure 2. Sample area for psyllid damage.

A. Loss of up to 25% of young leaves.

B. Loss of 51-75% of young leaves



2. PREDATOR IDENTIFICATION AND COUNTS.

Any apparent predators should be observed. For identification; collect the larvae and rear them through to adults. The British Museum has agreed to collaborate with us on the identification of predators. We strongly suggest that all collaborators send samples to the British Museum for consistency in identification. Samples can be sent as mounted specimens on cards or in alchohol. Please note with the sample that these identifications are part of the LPT network coordinated by NFTA. A small fee will be charged for each sample. Samples are to be sent to:

Laurence A. Mound
Keeper of Entomology
British Museum (Natural History)
Cromwell Road
London SW7 5BD
United Kingdom
Telegrams: Nathismus London SW7

The number of predators are to be scored empirically (0-3 scale) at the same time as the psyllid count scoring. One scoring can be done for each entry in each replication. If more than one predator is evident, please record each one separately.

<u>Predator Rating Scale</u>:

0- None
1- Rare
2- Common
3- Many

3. GROWTH MEASUREMENTS

Height and basal diameter (at 15 cm above ground level) of 8 trees per entry in each replication are to be measured before each cutting. Fodder harvest data (fresh weight in kg) must be recorded at each cutting for each entry in each replication, using a 2 meter hedge section. In one replication, separate the lea and small stem fraction (animal-edible fraction) from the wood fraction and weigh separately. A small sub-sample can be taken to provide data on percent moisture.

4. CLIMATIC DATA

Monthly rainfall and maximum/minimum temperatures must be recorded to coincide with the time of monthly psyllid counts. Dates of severe storms and drought-induced leaf drop must also be recorded.

5. OPTIONAL MEASUREMENTS

If convenient, additional studies can be done in the same trial and include the following:

5.1 Correlating empirical rating scale to actual counts.

This study can be done using the assessment technique developed by Stechman, et al. described in the Proceedings of the workshop of Biological and Genetic Control Strategies for the Leucaena Psyllid (Leucaena Research Reports No. 7 (2), 1987.)

5.2 Evaluation of forage quality.

Forage quality can be assessed by measuring the feed value (crude protein, crude fibre, etc.), mimosine content, IVDMD, and palatability.

PROGRESS REPORTS

All data must be sent to NFTA every 3 months (after each cutting). All collaborators are expected to submit 1-2 page research results and/or observations to be published in the LEUCAENA RESEARCH REPORTS. Please contact Dale Withington, NFTA Executive Secretary, for guidelines and deadlines. An annual site visit will be arranged by NFTA associates in the region.

SITE DESCRIPTION

Collaborator:
Address:

Location of Trial:

Latitude:
Topography (Slope, Aspect):

Longitude:

Elevation (m):

CLIMATIC DATA

Mean Annual Precipitation (mm):
Months with less than 50 mm of precipitation:

Mean Annual Temperature (C):
Minimum Temperature (C):

Maximum Temperature (C):

You will be asked to submit monthly rainfall and temperature (minimum and maximum) data during the extent of the trial.

SOIL DATA

Soil Type (USDA Classification):

Soil fertility:

You are expected to take soil samples at 0-20 cm and 20-50 cm for fertility anaylsis. Anaylsis should include Ca, Mg, K, Na, P, N, pH, Organic Matter, Extractable Al (KCl extraction), Texture. Please indicate method of anaylsis. Preferred anaylses include:

NH4OAc extraction, pH7 for Ca, Mg, K, Na
1:1 H2O for pH
Walkely and Black for Organic Matter
Olsen for P
1N KCl extraction for Extractable Al

EXPECTED DATE OF PLANTING:

cw10

LEUCAENA PSYLLID TRIAL NETWORK DATA

TRIAL NO._____ DATE PLANTED_____ DATE OF MEASUREMENT_____
MEASUREMENT (EGGS, NYMPHS, ADULTS, DAMAGE)_____

CLIMATIC DATA

MONTH	RAINFALL(mm)	MAX. TEMP (C)	MIN. TEMP. (C)

ENTRY	REP	1	2	3	4	5	6	7	8	MEAN
K8	1									
K636	1									
K376	1									
KX1	1									
KX2	1									
KX3	1									
K156	1									
#46568	1									
#33820	1									
L. collinsii	1									
K785	1									
G. sepium	1									
K8	2									
K636	2									
K376	2									
KX1	2									
KX2	2									
KX3	2									

12

K156	2										
#46568	2										
#33820	2										
L. collinsii	2										
K785	2										
G. sepium	2										
K8	3										
K636	3										
K376	3										
KX1	3										
KX2	3										
KX3	3										
K156	3										
#46568	3										
#33820	3										
L. collinsii	3										
K785	3										
G. sepium	3										

LEUCAENA PSYLLID TRIAL NETWORK DATA
GROWTH MEASUREMENTS

TRIAL NO._____ DATE PLANTED_____ DATE OF MEASUREMENT_____

HT = Height in meters
BD = Basal diameter in cm

ENTRY	REP		1	2	3	4	5	6	7	8	MEAN
K8	1	HT									
		BD									
K636	1	HT									
		BD									
K376	1	HT									
		BD									
KX1	1	HT									
		BD									
KX2	1	HT									
		BD									
KX3	1	HT									
		BD									
K156	1	HT									
		BD									
#46568	1	HT									
		BD									
#33820	1	HT									
		BD									
L. collinsii	1	HT									
		BD									
K785	1	HT									
		BD									
G. sepium	1	HT									
		DB									
K8	2	HT									
		BD									
K636	2	HT									
		BD									
K376	2	HT									
		BD									
KX1	2	HT									
		BD									
KX2	2	HT									
		BD									

ENTRY	REP		1	2	3	4	5	6	7	8	MEAN
KX3	2	HT									
		BD									
K156	2	HT									
		BD									
#46568	2	HT									
		BD									
#33820	2	HT									
		BD									
L. collinsii	2	HT									
		BD									
K785	2	HT									
		BD									
G. sepium	2	HT									
		BD									
K8	3	HT									
		BD									
K636	3	HT									
		BD									
K376	3	HT									
		BD									
KX1	3	HT									
		BD									
KX2	3	HT									
		BD									
KX3	3	HT									
		BD									
K156	3	HT									
		BD									
#46568	3	HT									
		BD									
#33820	3	HT									
		BD									
L. collinsii	3	HT									
		BD									
K785	3	HT									
		BD									
G. sepium	3	HT									
		DB									

LEUCAENA PSYLLID TRIAL NETWORK DATA
BIOMASS MEASUREMENTS

TRIAL NO._____ DATE PLANTED_____ DATE OF MEASUREMENT_____

ALL MEASUREMENTS MUST BE IN DRY WEIGHT (KGS) PER 2 METER HEDGE SAMPLE

ENTRY	REP	ANIMAL EDIBLE FRACTION	WOOD FRACTION	TOTAL BIOMASS
K8	1			
	2			
	3			
	MEAN			
K636	1			
	2			
	3			
	MEAN			
K376	1			
	2			
	3			
	MEAN			
KX1	1			
	2			
	3			
	MEAN			
KX2	1			
	2			
	3			
	MEAN			
KX3	1			
	2			
	3			
	MEAN			
K156	1			
	2			
	3			
	MEAN			
#46568	1			
	2			
	3			
	MEAN			
#33820	1			
	2			
	3			
	MEAN			

ENTRY	REP	ANIMAL EDIBLE FRACTION	WOOD FRACTION	TOTAL BIOMASS
L. collinsii	1			
	2			
	3			
	MEAN			
K785	1			
	2			
	3			
	MEAN			
G. sepium	1			
	2			
	3			
	MEAN			