Field Demonstration of the Use of *Metarhizium anisoplae* for Desert Locust Control Using the Release – Spray- Recapture Method

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Improving Pesticide Application Techniques for Desert Locust Control

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This report is prepared under GCP/INT/651/NOR, “Improving Pesticide Application Techniques for Desert Locust Control”, a collaborative project between FAO, the Centre National Lutte Antiacridienne, Nouakchott, Mauritania, and other countries of the EMPRES Western Region. The project is funded by Norway and executed by FAO.

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1 Introduction

In order to reduce the reliance on synthetic organic insecticides, the LUBILOSA (Biological control of Locusts and Grasshoppers) have developed and marketed an insecticide based on the fungus *Metarhizium anisoplae var acridum* Gams & Rozsypal (Deuteromycotina: Hyphomycetes). This fungus is marketed as "Green Muscle", and is available in dry powder and oil flowable formulations.

One of the objectives of the EMPRES programme is to promote the use of more environmentally friendly control methods for Desert Locust such as "Green Muscle". However, one of the limitations has been the absence of field populations of Desert Locust on which to undertake large scale trials.

Under the auspices of the project "Improving Pesticide Application Techniques for Desert Locust Control", a workshop was held in Mauritania from 17th – 30 November 2001. During this workshop – Improved Spraying Techniques and Novel Survey Methods in Desert Locust Control – a session was held on the use of "Green Muscle" under field conditions. The aim of this work was to demonstrate to participants – from India, Mauritania, Pakistan, Sudan and Yemen – how to use and evaluate the efficacy of "Green Muscle" (GM) under field conditions, in order that when the opportunity arises they will be able to undertake large scale field trials in their own countries to promote the uptake of this product for Desert Locust control. An expert from the LUBILOSA project (Mr Kpindou) led this section of the workshop.

The specific objections of this work were to demonstrate :

- how to prepare the formulation for use
- the application of GM
- sampling methods
- to measure the persistence of GM in the field

in order that the participants could execute this work in their own conditions using a standard technique.

2 Methods

As no field populations of Desert Locust were present at the time of the demonstration, insects from the large scale rearing facilty at Nouakchott were used as the target. (A large scale rearing facility at Akjoujk had been established to produce insects for this work, but unfortunately the insects at Akjoujt were not suitable due to their stage of development. Thus insects from Nouakchott were used. This meant that the longer transport required to the field site resulted in some mortality as a result of stress).

The method used was that these reared insects were released into the target area a few minutes before application. After the plot had been sprayed, at various times after spraying samples of insects were captured for assessment of biological effect. This method does not permit a estimation of population control, as released insects are generally preyed upon very quickly after release.

2.1 Site Description

The area used or the trial was a wadi located 50 km (19 37 759 N 14 4 825 W) which was a run off area for water coming from the hills north west of Akjoujt. Soil was sandy-clay. Vegetation cover (estimated at 70%, mean plant height 0.4 m with a leaf area index of about 4) was largely *Farsetia ramossissima.* Other species present included *Corchorus depressus, Heliotropium bacciferum, Psorellea plicata, Indigofera sp, Gisekia pharmacoides, Panicum turgidum, Maerua crassifolia, Calotropis procera,Capparis decidua, Acacia raddiana* . (Photo 1).

Photo 1

2.2 Germination Test

A sample of the formulation was analysed for spore germination before and after the treatment. The spore suspension is diluted with diesel in order to adjust the concentration of spores to facilitate counting. Two or three drops of the diluted suspension were placed in each of three petri dishes which contained Sabouraud Dextrose Agar (SDA). The dishes were incubated at 26 ^{o}C for 20 hours. The dishes were examined under a microscope (40X) and the number of spores germinated (ie showing a germ tube) and non germinated were counted.

Repititions	**Before Application**			**After Application**		
	Germinated Spores	Spores Non Germinated	Total	Germinated Spores	Spores Non Germinated	Total
I	118	11		126	20	
II	122	6		103	19	
III	93	8		94	9	
Mean	111	8.33	119.33	107.66	16	123.66
% germination	**93.02**			**87.06**		

2.3 Application Parameters

Two Micron ULVA+ units were used simultaneously

Formulation : Oil Flowable (OF)
Dosage : 50 g spores/ha
Volume Application Rate : 2 l/ha (0.1 l OF + 1.9 l diesel)
Equipment : Micron ULVA+
Flowrate : 70 ml/min
Track Spacing : 6 m (marked by flagmen)
Number of Batteries : 5
RPM : Unit 1 6324; Unit 2 6341

Oil sensitive papers (28) were place throughout the plot in order to monitor the number of droplets collected.

2.3.1 Application Conditions

	Start	**End**
Time	9h36	9h48
Temprature (0C)	25	25
%RH	23	23
Windspeed ms^{-1}	4.65	4.65

Wind direction throughout the spraying period was 70 – 90 ^{o} relative to the spray track. Conditions were generally good for the application.

About 15 minutes before spraying, approximately 5000 Desert Locusts (a mixed population structure of $L_2 – L_5$) were released into the plot.

2.4 Sampling Methods

2.4.1 Insects present in the plot at the time of treatment.

Two hours after treatment 80 insects ($L_2 – L_5$) were collected and divided into 4 cages (20 per cage – cage size 25 cm x 25 cm x 30 cm). The cages were transported to the laboratory for monitoring.

Three days after treatment, a further 80 insects were collected from the plot and treated as above.

Six days after treatment, no insects could be found in the treated area, although 2 were found the following day and collected.

2.4.2 Persistence of GM Activity

To study the persistence of GM, 3 cages (constructed from mosquito netting, 70 cm x 70 cm x 80 cm) were placed in the plot after treatment. In each cage 20 insects ($L_2 - L_5$) from an untreated population were released. These insects were left in the cages for 72 hours, after which they were collected and removed to the laboratory for observation. This was done 5 times – immediately after application (0 DP), and 3, 6 9 and 12 days after application). For each time, the cages were moved to a new point on the plot.

2.4.3 Untreated Controls

Three cages containing 20 insects each were used as controls. They were kept under the same conditions as the insects sampled from the field. They were followed for 15 days, and were used as a comparison between treated and untreated for 0 and 3 days after treatment for those insects present during spraying, and for the persistence samples for 0 and 3 days after application.

However, it became clear that the mortality in the control was very high. This can largely be ascribed to the effect of the long transport and additional handling of the insects. A new control was established for insects exposed to the plot 6, 9 and 12 days after treatment, using insects from the rearing facility at Akjoujt.

2.4.4 Data Collected

Temperature and relative humidity inside and outside the laboratory were noted at 2 hourly intervals during the day.

Cages were checked daily for mortality, and dead insects were removed and placed in petri dishes (see below). Cages were cleaned and fresh food placed in regularly.

The dead insects were allowed to dry in petri dishes for 24 hours. They were then placed on a piece of moist paper in the same dish and left for a further 24 hours. Those insects which were infected with the fungus clearly showed sporulation after this time.

3 Results

The average number of droplets counted on the cards was 16.9 per cm2. This is lower than expected and probably results from the fading of the deposit. For operational reasons, it was not possible to analyse the oil sensitive paper until some days after the treatment, and although diesel marks the paper clearly, the stain does fade after a time. It is likely that this has led to an underestimate of the drops actually deposited.

3.1 Mortality – Insects present in Plot at Time of Application

3.1.1 Insects Collected 2 hours after treatment

15 days after treatment, the insects which were present during spraying and collected 2 hours after treatment showed a mortality of 92.5 %. However, control mortality was high at 44..3%. (Figure 1). As discussed above, this high mortality probably resulted from stress due to transport. Fifteen days after treatment, 37% of the insects were showing signs of sporulation.

3.1.2 Insects Collected 3 days after treatment

Figure 2 presents this data. It should be noted that the control data used in this will include those insects which had died as a result of stress during transport. However, for those insects collected three days after treatment (DAT), only those that survived for after release will have been collected. However, by 8 DAT the mortality in the treated insects began to exceed that of the controls, and reached 77.5% by 18 DAT. Sporulation was evident from 7 DAT and reached 48.5% by 18 DAT.

3.2 Persistence of GM

3.2.1 Insects Exposed Immediately After Application (0 D)

The total mortality of the insects exposed to the deposit immediately after treatment is lower than those exposed during treatment (Fig 3), reaching 57%. However, in terms of sporulation, there was little difference between those on present during treatment and those exposed immediately after (about 37% in both cases).

3.2.2 Insects Exposed 3 Days After Application (3 DAT)

The results are similar to 0 D; with the maximum mortality reaching 59% and 37% showing signs of sporulation 21 DAT – which is equivalent to 18 days after first exposure of these insects to the treated plot, and 15 days after removal from it. (Figure 4).

3.2.3 Insects Exposed 6 Days After Application (6 DAT)

A new control batch was used in at this time. Mortality reached 51% 24 DAT, while sporultation had fallen to 14% (Figure 5).

3.2.4 Insects Exposed 9 Days After Application (9 DAT)

Figure 6 illustrates that the difference in mortality between the control and treated insects was about 10% (26% in the control and 36% in the treated 27 DAT). Interestingly, about 10% of the insects showed signs of sporulation.

3.2.5 Insects Exposed 12 Days After Application (12 DAT)

There was no difference between the control and treated insects. No sporulation was evident 30 DAT. (Figure 7).

The results are summarised in Figure 8.

3.3 Temperature and Relative Humidity

Temperature and relative humidity were recorded at 2 hourly intervals in both the laboratory (where the insects were kept after collection) and exterior (as an indication of the conditions in the field). These data have been analysed on a frequency basis as an indication of the temperature and relative humidity variation throughout the daytime (Figure 9 – 12). It is clear that there is less variation in the laboratory, where over 75% of the readings were between 25 ^{o}C and 29 ^{o}C; this compares to 50% for the field readings. Similarly, the relative humidity stayed more constant in the laboratory, with 85% of the readings between 21 and 24% RH, compared to 65% for the field.

4 Discussion

Figure 8 is a summary of the results. In terms of mortality it is difficult to draw any conclusions due to the high control mortality as a result of stress induced by transport. The failure of the culture in Akjoujk to provide sufficient locusts meant that insects had to be transported from Nouakchott.

There is however, a clearer picture in terms of insects showing signs of sporulation (and therefore infected with the fungus). For those insects subjected to direct spray and removed 2 hours after treatment, 37% of the insects were confirmed as being infected by the fungus. This increased to 48% when the insects were left on the treated plot for three days. Three days after treatment however, this had fallen to 37%, and continued to fall to 0 at day 12 after application. This therefore indicates that, under the conditions throughout this work, the biological effectiveness of the fungus had disappeared by day 12, presumably as a result of spore degradation by sunlight and temperature.

It is not possible to exclude the fact that the deposit obtained from the application was comparatively low in terms of drops per cm^2. The data from the oil sensitive paper is unreliable due to the possibility of the stains fading thus resulting in undercounting.

5 Conclusions

The nature of this work was to demonstrate procedures in the use of Green Muscle, such that the participants in the workshop felt confident in undertaking field trials in their own countries. As such it was a very useful exercise. It was not designed to give a definitive answer to the biological effectiveness of the fungus; that requires large scale field trials on natural populations.

A number of points emerged from this work:

Due to the viscosity of the OF formulation, it required dilution with 1.9 l of diesel to make a sprayable suspension. However, discussions indicated that this was an unusually high quantity, and normally it could be sprayed at 0.5 – 1 l/ha total application volume. It is probable that this was an exceptionally viscous batch.

Mortality was clearly attributable to the fungus, and was clear on all the nymphal stages used ($L_2 – L_5$).

A clear reduction in the biological activity of GM was seen from the data of sporulation. No insects exhibited sporulation which were exposed to the deposit 12 days after application.

6 Acknowledgements

We would like to thank IITA for allowing Mr Douro Kpindou to lead this work.

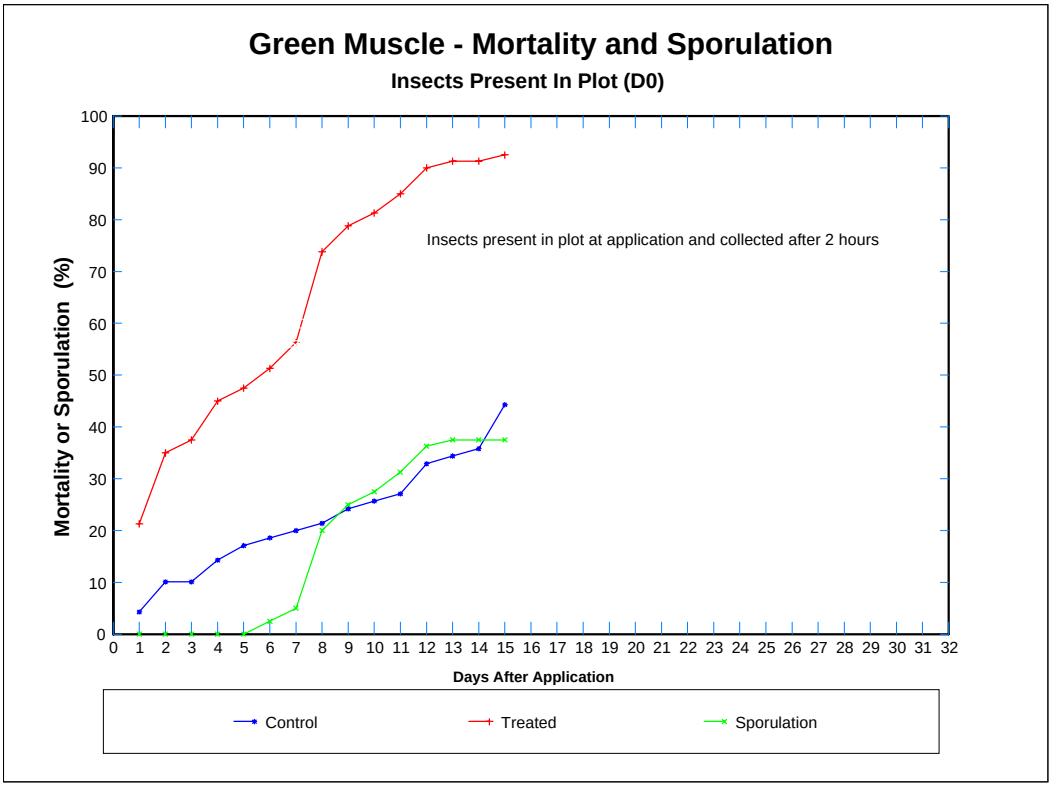
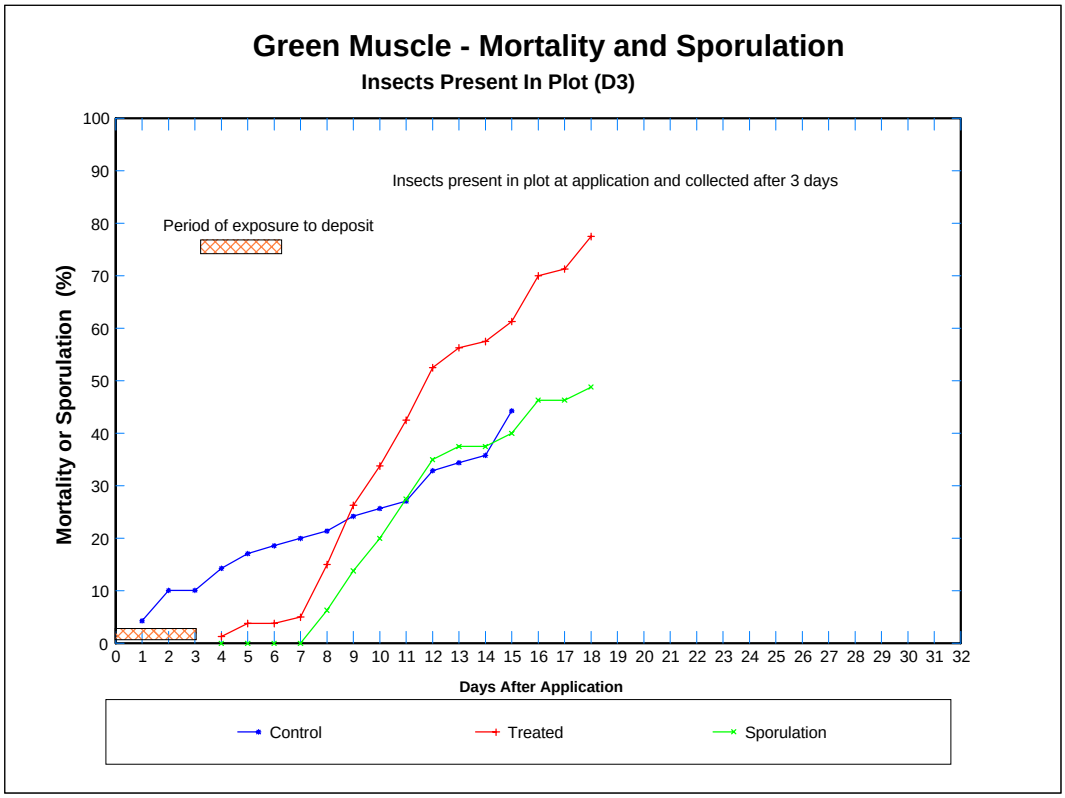


Figure 1



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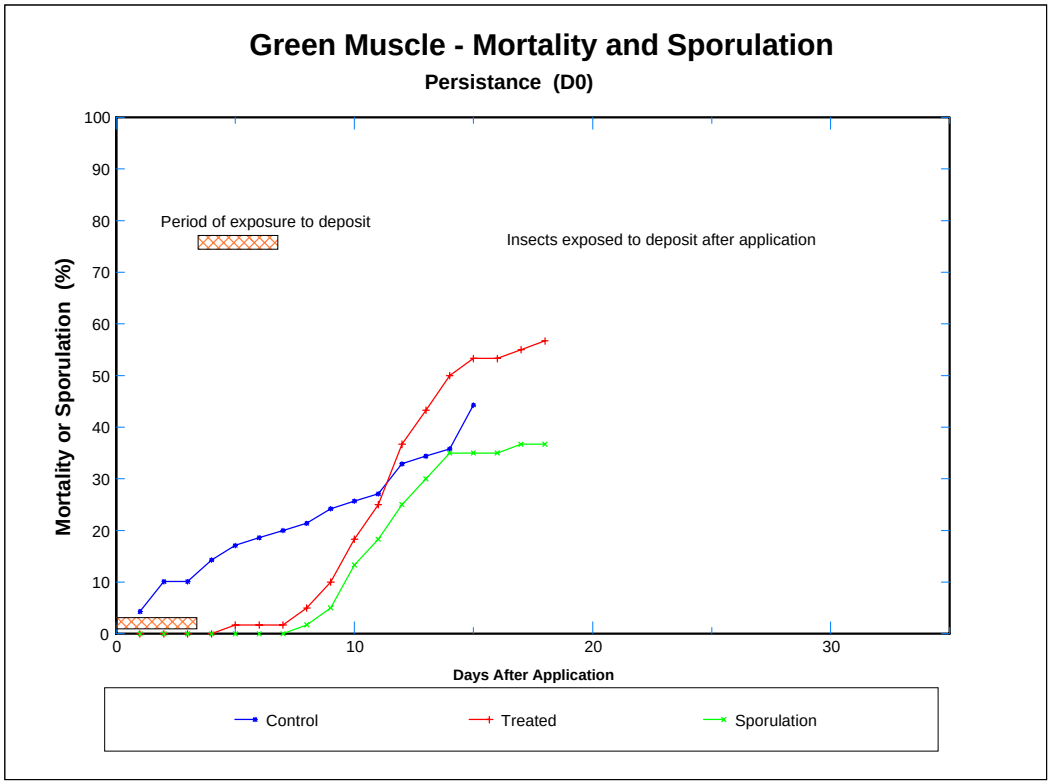

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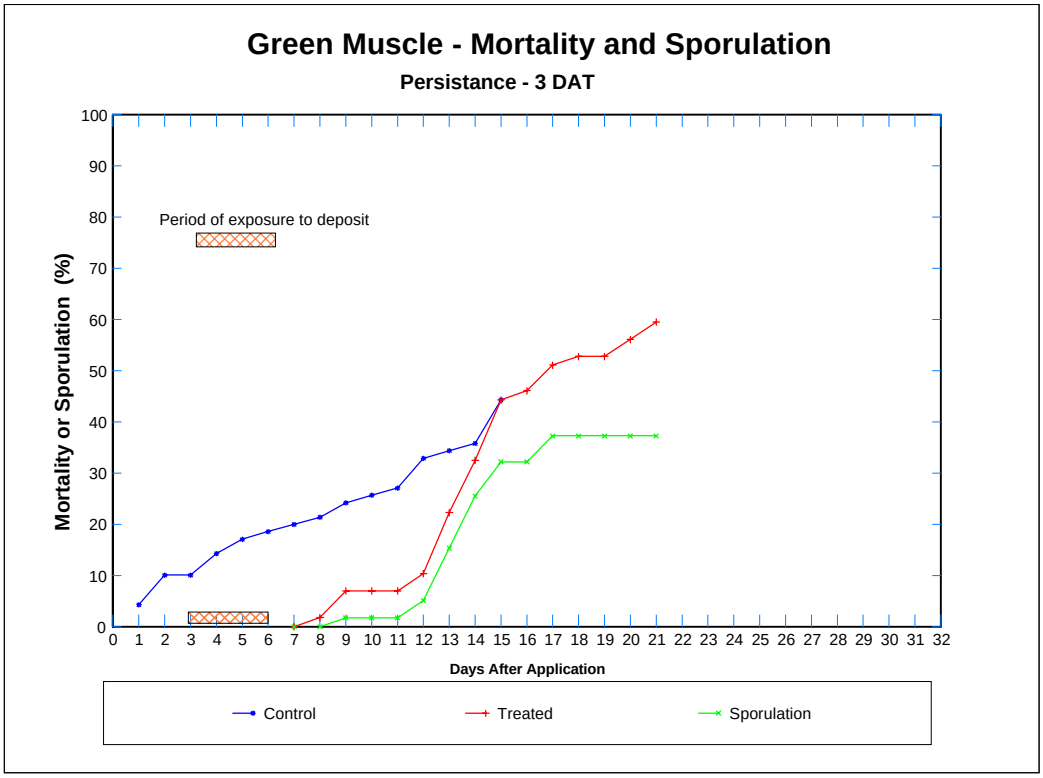

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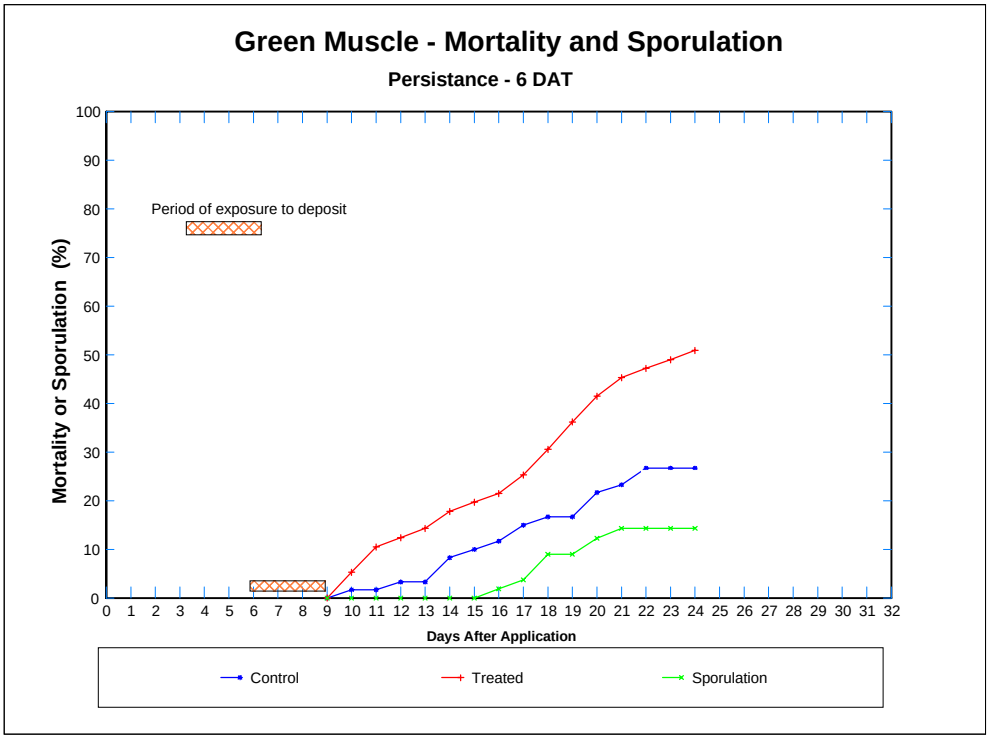

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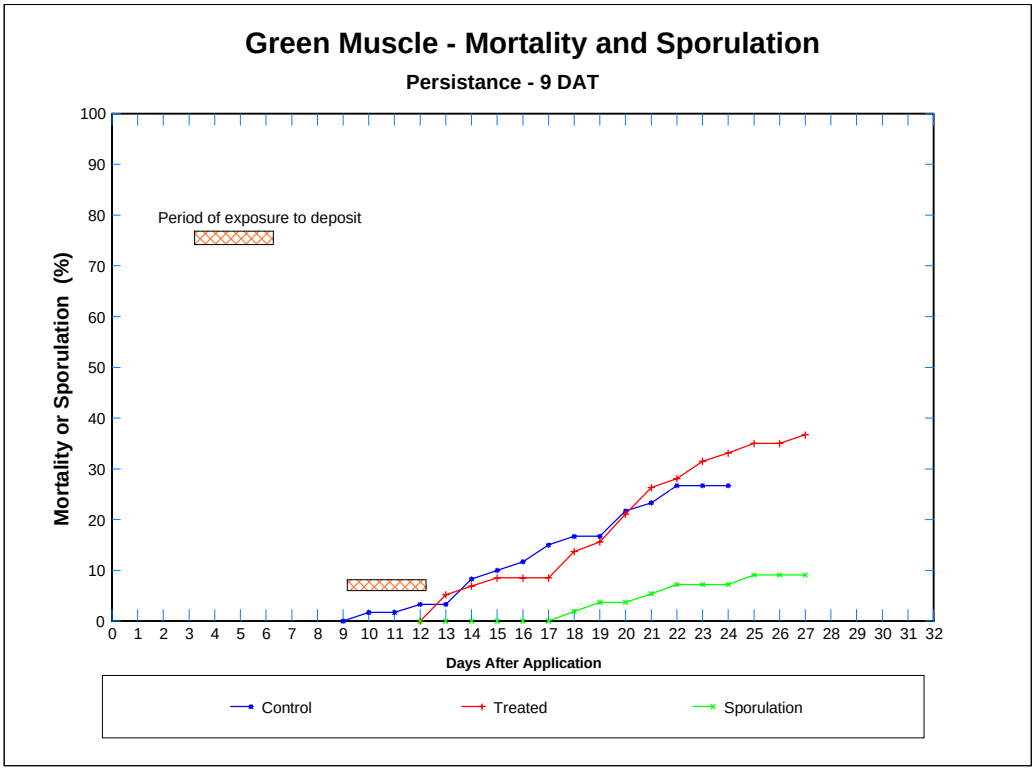

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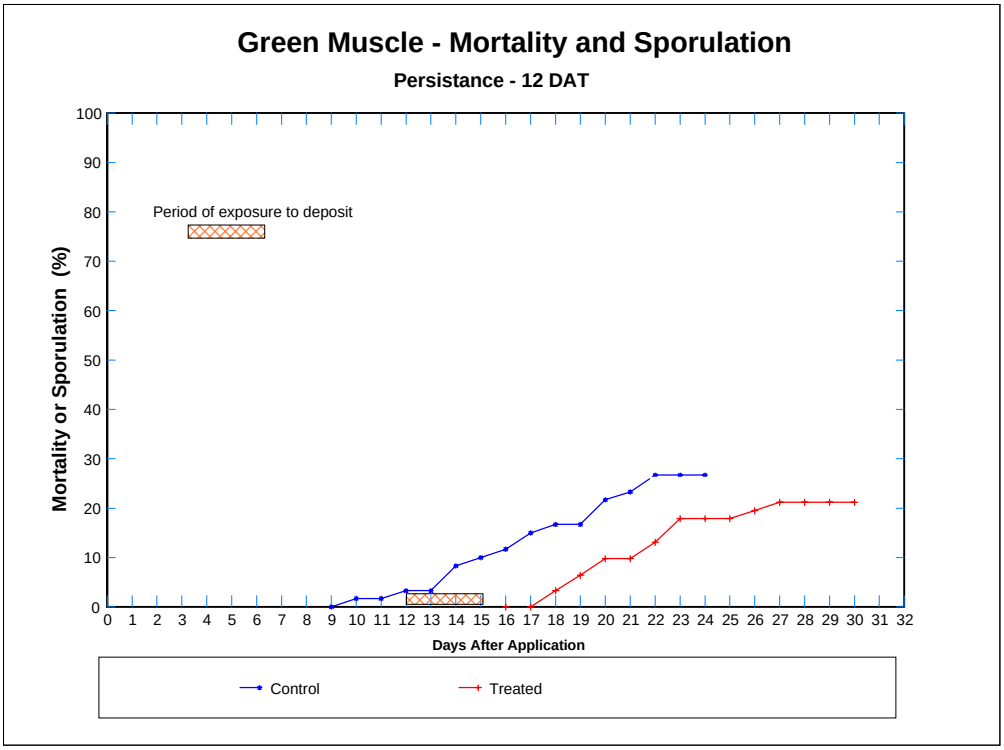

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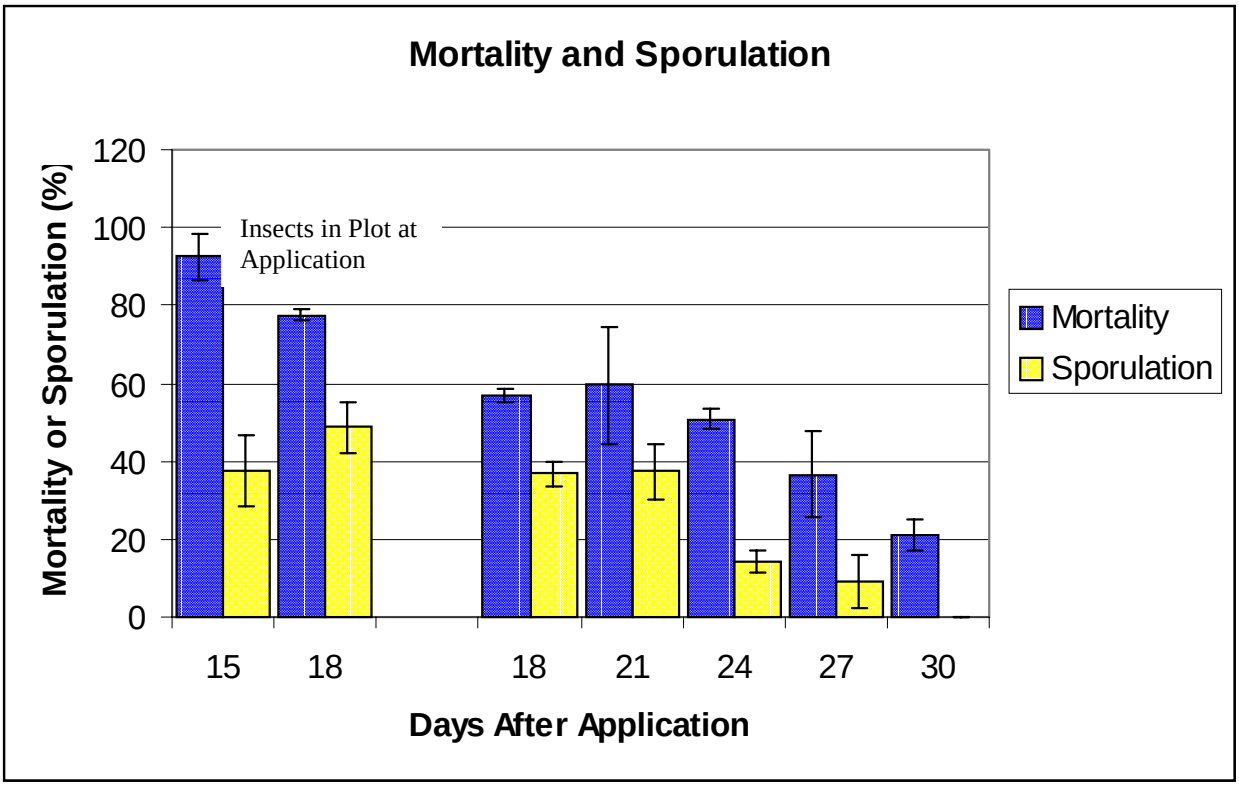
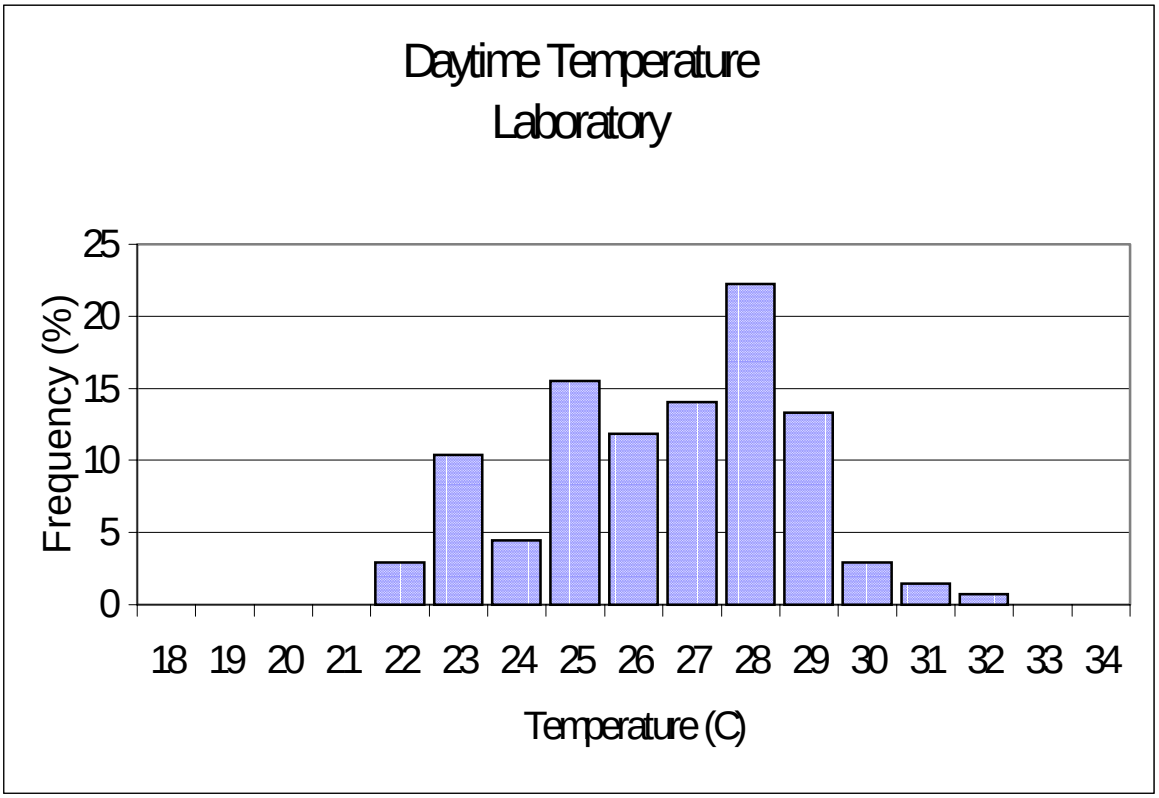


Figure 8



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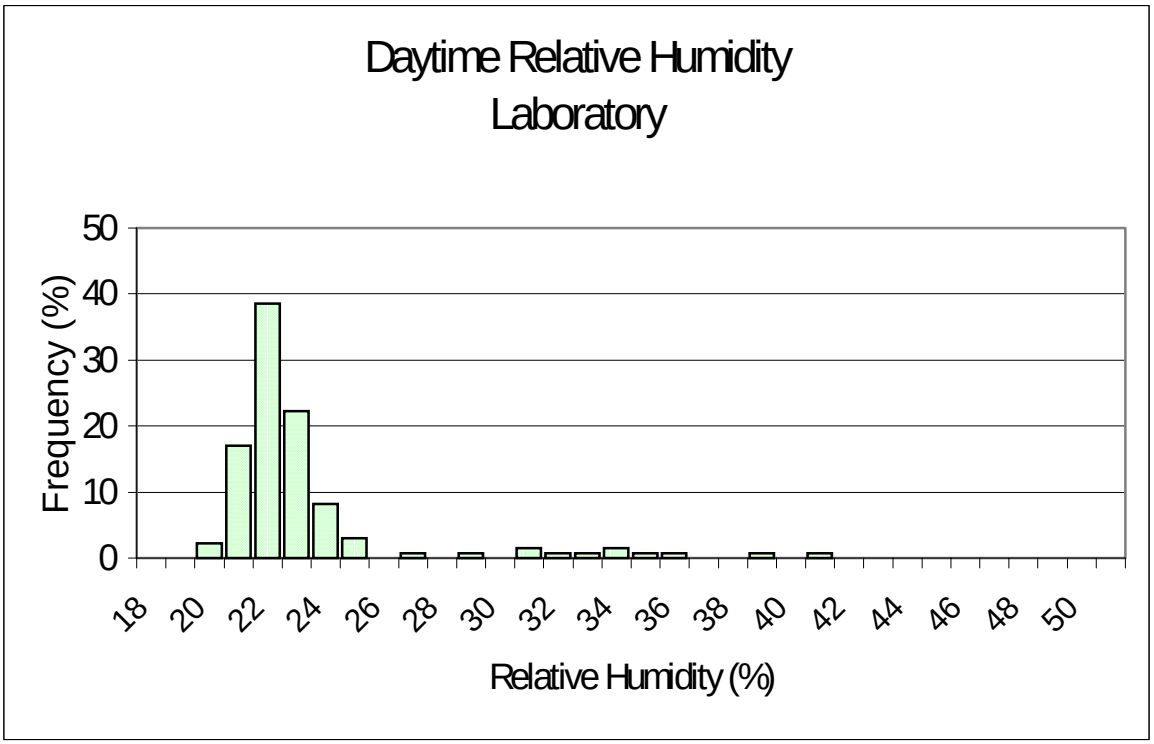

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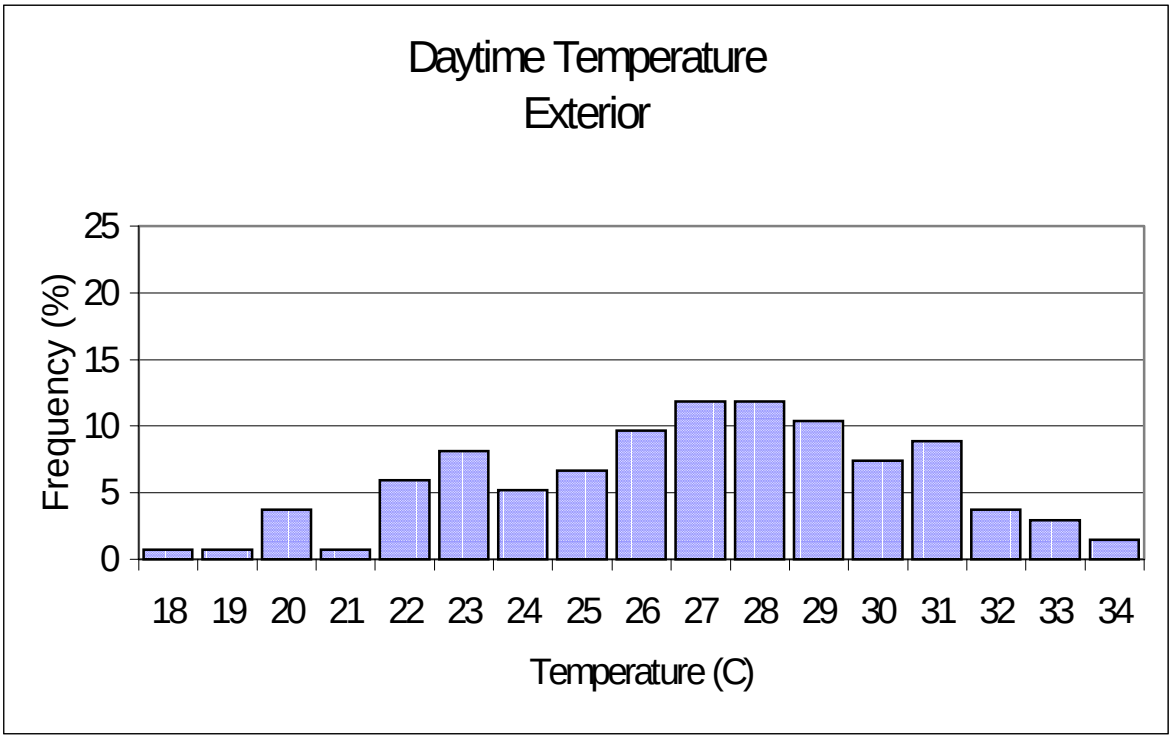

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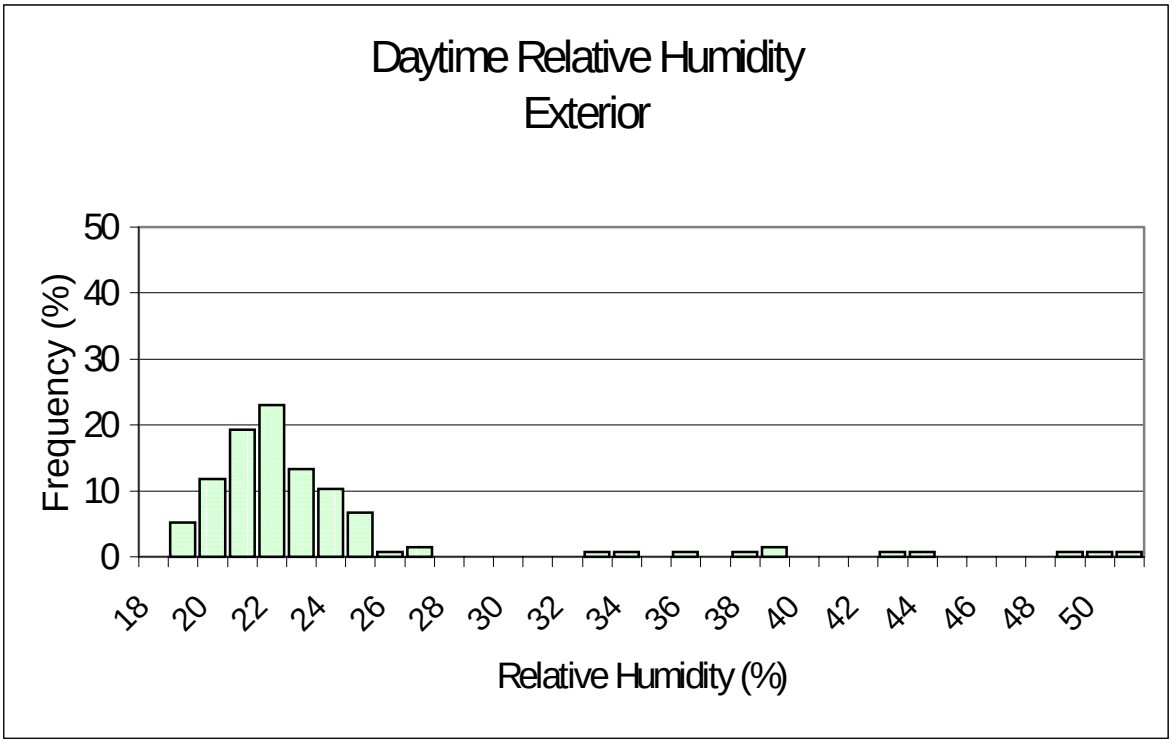

Figure 12