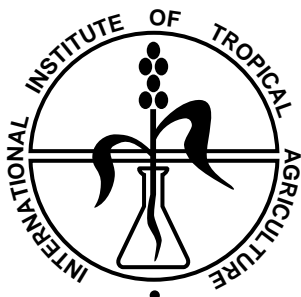
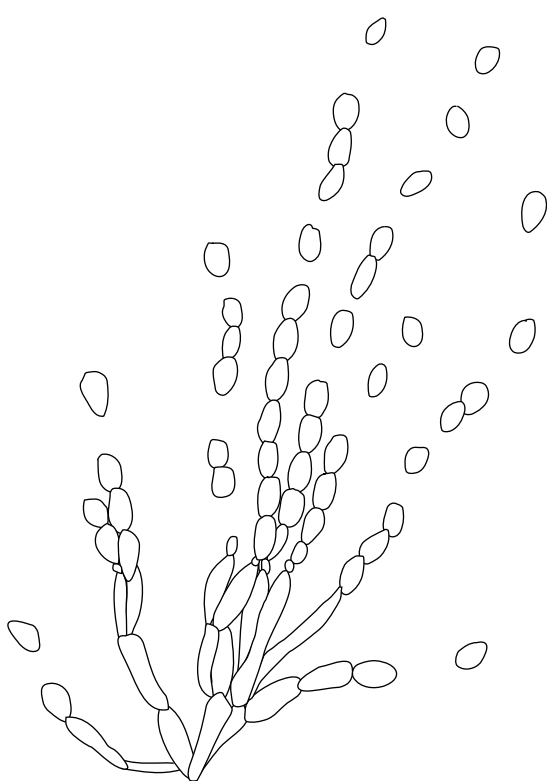


Green Muscle

User Handbook

1999



LUBILOSA (Lutte Biologique contre les LOcustes et les Sauteriaux)

Biological Locust and Grasshopper Control Project

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1. Introduction

The LUBILOSA project was initiated in 1989 in response to environmental concerns over the heavy use of chemical insecticides to control locusts and grasshoppers during the 1986-89 plagues. Funded by the governments of Canada, the Netherlands, Switzerland, Britain and the USA, the project is executed by the UK (Ascot) laboratory of the International Institute of Biological Control (CAB International), the International Institute of Tropical Agriculture from its Benin station (Cotonou), INSAH (part of CILSS) in Bamako, Mali, AGRHYMET/DFPV (part of CILSS) in Niamey, Niger and GTZ (Deutsche Gesellschaft für Technische Zusammenarbeit, Eschborn, Germany).

LUBILOSA is the acronym of the French “**LU**tte **BI**ologique contre les **LO**custes et **SA**uteriaux” - biological control of locusts and grasshoppers.

The project focuses on the use of beneficial micro-organisms (pathogens) as biological control agent for grasshoppers and locusts. These insects were considered to be too mobile and to reproduce too fast to be readily controlled by a classical biological control approach using for example, parasitic hymenoptera. An augmentative approach needing regular application of the control agent was adopted. Beneficial micro-organisms have the advantage that they can be produced in artificial culture in large quantities and be applied using ordinary spray equipment.

Micro-organisms and microbial control agents have not been fully exploited in western cultures. Fungi in particular have always been perceived as rather unreliable and needing humid conditions to work well. One of the principal successes of the LUBILOSA project has been to show that the requirement for high environmental humidity can be avoided by spraying fungal spores in oil. Even under desert conditions Green Muscle® can be used to kill locusts.

Green Muscle® consists of spores of the fungus *Metarhizium flavoviride* Gams & Rozsypal (Deuteromycotina: Hyphomycetes). The *M. flavoviride* strains used in Green Muscle® kill locusts and grasshoppers. Formulations consist of dry powder (TC formulations) or oil miscible concentrate (OF formulations). Green Muscle® is applied as an oil suspension (SU) using Ultra Low Volume (ULV) spinning disk spray equipment. Although it is used like a chemical insecticide, it has no “knock down” effect. Grasshoppers and locusts take 6 to 10 days to die; however, they are slowly incapacitated during this period, eating and moving much less than healthy insects after only 3 or 4 days. A big advantage over many common insecticides is the rather long persistence of the spray residue in the treated vegetation. Depending on environmental conditions the spores can survive several weeks and continue to be a source of infection to healthy grasshoppers. Thus one application per agricultural season is normally sufficient, while repeated treatments often necessary with modern synthetic pesticides and .

Green Muscle® is safe to use compared with many chemical pesticides and can be used near water courses. Under field conditions eco-toxicological studies demonstrated no negative effects on non target insects. Tests carried out by LOCUSTOX, Senegal by Agriculture Canada and by LUBILOSA demonstrated that neither birds nor fish were affected by the fungus. Similarly, there were no infections in beetles (Coleoptera) or bugs (Heteroptera). Only in full exposure tier I laboratory tests, hymenoptera including some parasitic wasps and honey bees were affected by the fungus. *Metarhizium flavoviride* has been extensively safety tested by an independent laboratory. No strains caused infections in mammals, and the strains recommended for use gave no adverse reactions. However, normal precautions should be taken to avoid inhalation and contact with eyes to avoid allergic reactions.

The project started in 1989 and is now in its third phase; as well as developing and field testing Green Muscle®, the project has published numerous scientific papers (a full list is available on request), has given many presentations at meetings and has been involved in training scientists. The development of Green Muscle® involved collecting fungus isolates through a network of collaborators in Africa; selecting the best fungus to use; developing a production method; finding out how to store the fungus; checking its safety both to human operators and to the environment and non-target organisms. Extensive field tests and ecological studies were carried out. During the current phase the project is emphasising participatory trials with farmers, NGOs and national programmes. Socio-economic studies are carried out to define the role Green Muscle® can play in grasshopper and locust control. All field studies were carried out in close collaboration with plant protection services or Non Governmental Organisations in Benin, Ghana, Niger, Mali, Mauritania, Senegal, Burkina Faso, Madagascar, Spain, South Africa and Australia.

2. Field results

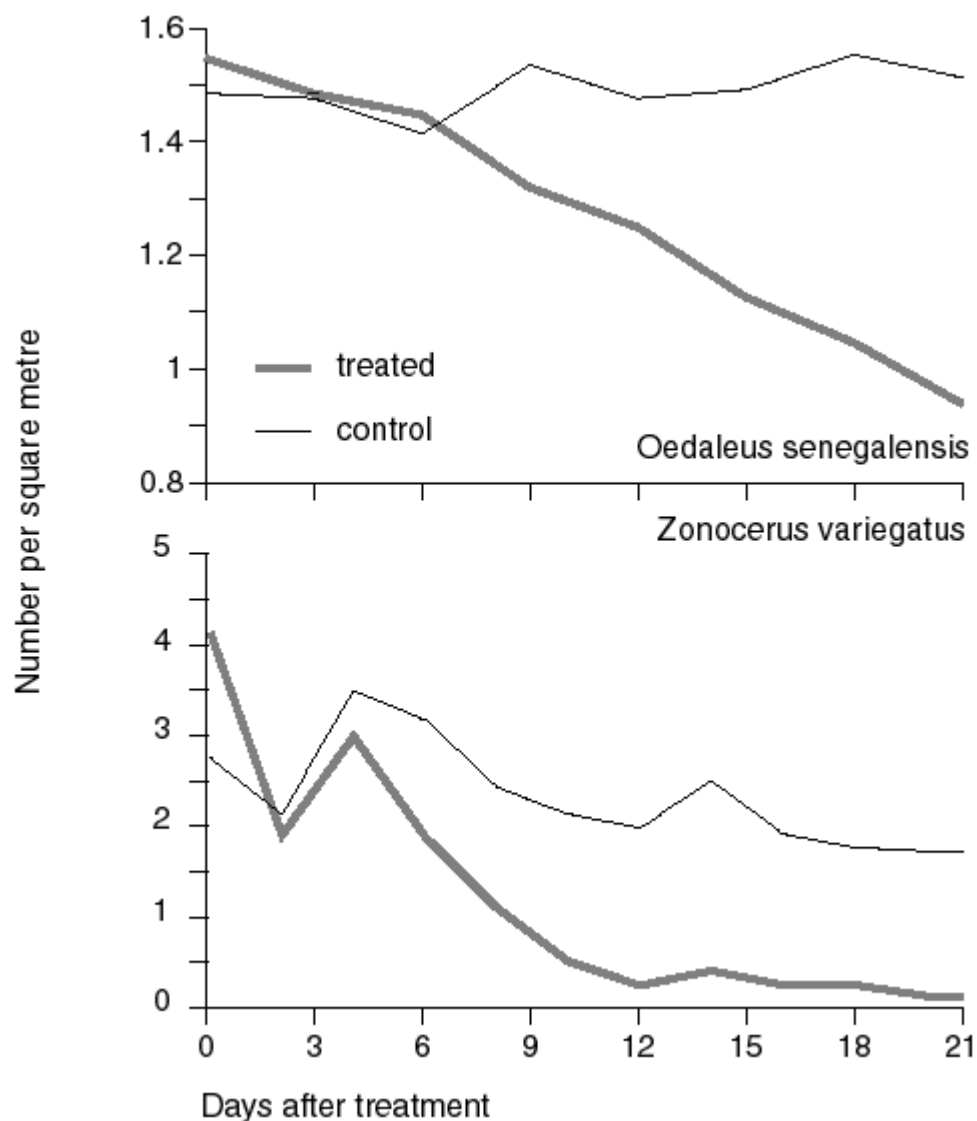
Green Muscle® is effective against all instars, nymphs and adults of grasshoppers and locusts.

The first good results with Green Muscle® were obtained with non-migratory grasshoppers such as the Variegated Grasshopper, *Zonocerus variegatus*, and the Rice Grasshopper, *Hieroglyphus daganensis*, in Benin and the Wingless Grasshopper, *Phaulacridium*, sp. in Australia. With increasing plot size and improved sampling plans good results have been also obtained with the more mobile Sahelian grasshoppers such as *Kraussella amabile*, *Kraussaria angulifera* and *Oedaleus senegalensis*.

Trials against Desert Locust hopper bands in Mauritania were also successful in eliminating the bands, and trials against Brown Locust in South Africa and Plague Locust in Australia were promising.

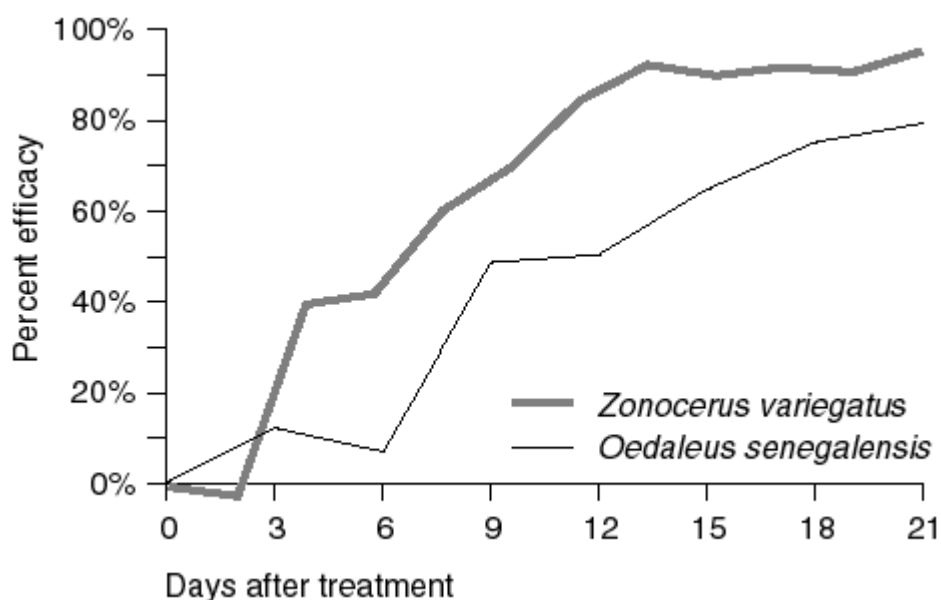
Data from field applications against *O. senegalensis* carried out in 1995, 1996 and 1997 in Niger on 50 and 800 ha plots using vehicle mounted and aircraft mounted spray equipment demonstrate the decline of the population treated with the strain IMI 330189 compared with the non treated control. Similar results were obtained from a field trial on *Z. variegatus* carried out in 1994 in Benin using the strain I 91609. The graph below also shows that *Z. variegatus* is killed faster than *O. senegalensis*.

Grasshopper kill after application of Green Muscle®



Insect counts from the treated plots corrected for the counts from the control plots using Henderson & Tiltons formula are used to express percent control efficacy. It takes 14 days to achieve the maximum efficacy against *Z. variegatus*. Control of *O. senegalensis* however, takes more time.

Percent efficacy after application of Green Muscle®



3. Storage

Green Muscle® is normally supplied as a dry powder (TC) formulation mixed with silica gel desiccant in an air-tight and moisture-proof foil sachet.

The package should be kept sealed until needed; if the package is punctured or inadvertently opened, close the whole package in a plastic bag or foil wrapper and seal hermetically.

The package should be kept as cool as possible. The following is a rough guide to expected shelf life (>80% spore viability) at different temperatures:

Temperature (°C)	4°	26-32°	40°	50°
Time of storage (months)	>42	12	1	1 week

Green Muscle® is also supplied as an oil miscible flowable (OF) with a storage life of at least one season .

Once diluted to the field concentration Green Muscle® should be used within one week.

Whatever the circumstances, a simple germination test (see Appendix) allows you to check spore viability.

4. Formulation

Green Muscle® is designed for use with ULV sprayers, and should be suspended in an oil formulation (SU). It is delivered as dry spore powder technical concentrate (TC) or as an oil-miscible flowable (OF).

Make up the formulation the day before application wherever possible. However, formulations made up with good quality, dry, oils should keep for several weeks. The quality of the formulation can always be checked by carrying out a germination test

Technical concentrate

For the use of TC, make up the formulation by mixing the powder to a smooth paste in a small quantity of Shellsol T or kerosene inside the sachet. To avoid the creation of airborne clouds of spores, add liquid to the spores and not vice versa. Dilute the paste to the required volume with the formulation oils. Remove the sachet containing silica gel from the formulation.

AVOID INHALING SPORE DUST; ALWAYS WEAR A FACE MASK TO MAKE UP FORMULATIONS (risk of allergic reaction).

AVOID RISK OF EXPLOSION WITH CLOUDS OF SPORE DUST.

Two formulations which work well for the dry spore powder are:

50:50 Shellsol T: Ondina

70:30 kerosene: peanut oil

The diluted formulation is referred to as an SU oil suspension.

Diesel can be used to replace kerosene, and maize oil in place of peanut. However, with unlabelled ingredients, it is better to check that each batch is not damaging to the spores. Do this by carrying out a germination test (see Appendix).

A reasonable volume application rate is 0.5 - 2 l/ha. The spore application rate is 100g/ha, so make up 100g in 0.5 - 2 l.

Oil micsible flowable concentrate

If applied at volumes below 2 l/ha, oil-miscible flowable (OF) can be diluted with kerosene or diesel only (See product lable).

5. Application

Apply Green Muscle® at a rate of 0.5 - 2 litres/ha according. Some guidelines for different hand-held spinning disk sprayers are given below. However, you should carefully calibrate your spraying equipment on-site. See LUBILOS Technical Bulletin #4 for more details. For the calibration of vehicle mounted sprayers and aerial spray equipment please refer to LUBILOS Technical Bulletin #4 and to the manufacturer's manual.

Choice of restrictor

Sprayer	Flow rate (ml/min)	Restrictor
Ulva + or Micro Ulva	60	red
	45	orange
	30	yellow
Turbair XJ	60	1.3
Berthoud C5, C8	60	yellow
Ulva 8 or Ulva 16	60	yellow

Volume application rate	at 60 ml/min	at 45 ml/min	at 30 ml/min
Track spacing	Volume		
5m	2 l/ha	1.5 l/ha	1 l/ha
10m	1 l/ha	0.75 l/ha	0.5 l/ha

Use as narrow a track spacing as practical in order to achieve an even application (ca. 5m for hand held sprayers). Always check for adequate droplet coverage in unfamiliar spraying conditions using ultra-violet tracer or oil-sensitive cards. Although Green Muscle® has a good residual action, its most efficient mode of action is through direct contact. Spray directly at insects themselves as much as possible. When treating hopper bands or milling/roosting swarms ULV mist blowers should be considered to achieve higher direct impact. Only carry out ULV application in wind speeds of 1 to 4 m/s.

6. Monitoring results of application

Before application mark out one square metre squares. Count grasshoppers in at least 20 squares before application and twice per week afterwards; the counts should decrease after about 10 days.

It is useful to keep grasshopper samples both from the treated sites and from a non treated site in cages. The comparison of mortality lets you check that the application has worked properly. Protect cages from attack by ants. Remove cadavers, clean the cages every day and provide fresh food. If kept in the shade the insects will die faster in cages than in the field. Plastic water bottles with the bottom replaced by a screen attached with a rubber band are very suitable for grasshopper incubation.

Cadavers placed on moist substrate will sporulate after a couple of days, appearance of

green conidia confirms *M. flavoviride* as the cause of death. However, cadavers do sometimes only sporulate internally.

You can look for dead grasshoppers in the field, but don't be surprised if you don't find many because they are rapidly scavenged by ants or taken by predators.

It is important to monitor the full effects of Green Muscle® at the field site for several weeks.

7. Identification and Quality Standards

The genus *Metarhizium* belongs to the order Hyphomycetes in the subdivision Deuteromycotina. The deuteromycete fungi lack a sexual stage in their life cycle so the concept of species can only be used loosely with these fungi.

Within the genus *Metarhizium* the situation is even less clear. The 'species' *Metarhizium anisopliae* is large and varied. The 'species' *Metarhizium flavoviride* has three groups. The isolates which attack grasshoppers and locusts fall into one of these groups. Biochemical means are needed to distinguish between different isolates and between the different groups.

Strictly, the spores should be referred to as "conidia". The conidia are green spores and are produced on long chains (see cover). Other Hyphomycete fungi in the order may have white spores (*Paecilomyces*, *Beauveria*), black, brown or yellow (*Aspergillus*), or green or pink (*Penicillium*, *Nomurea*) (see LUBILOS Technical Bulletin #2).

The LUBILOS project holds a collection of about 150 isolates of *Metarhizium* from grasshoppers and locusts. These have been screened against the desert locust, and the most virulent examined more closely. The following are the isolates most widely tested:

IMI 330189 isolated from *Ornithacris cavroisi* from Niger
I91-609 isolated from *Zonocerus variegatus* from Benin.

Technical Specifications of Green Muscle®

Viability at packing:	>90%
Moisture content at packing:	<5%
Active ingredient (<i>M. flavoviride</i> spores):	>99%
Particle size (diameter):	<60µm
>99.9%	
Spore density:	5x10 ¹⁰
spores/g	

Virulence: 95% mortality in adult Desert Locusts at 5x10⁴ spores per insect in oil at 30°C within 5 days. Spores should have a light green colour, be unclumped and suspend readily in the formulation oil. Batches of spores which appear grey and clumped must be checked for germination before use.

8. Production

Green Muscle® is still produced at the LUBILOSA pilot production unit at IITA Cotonou in an appropriate technology process based on production of spores on rice in plastic bowls. Green Muscle® is produced at an industrial scale by Biological Control Products SA (Pty) Ltd. (BCP), for South and East Africa, and Madagascar. Production will soon be started by Natural Plant Protection (NPP) / Calliope for West Africa.

Disposal

Excess Green Muscle® may be returned to IITA for disposal, or autoclaved, or burned. Spores in kerosene can be poured over a flammable substrate such as paper or straw and ignited.

9. Relevant regulations

Quarantine - import/export

Green Muscle® is a live biological agent. Its import into any country must follow the FAO code of conduct on the importation of exotic biological control agents. This normally involves the issue of a permit by national plant protection or quarantine services. In Africa, the service will then inform the Inter-African Phytosanitary Council of the OAU.

Application

The technology is so new, that few countries have regulations specifically covering the application of biopesticides. The use of Green Muscle® has been approved by regulators using the current Canadian guidelines. Since 1996 Green Muscle® has been included on the FAO list of recommended products for grasshopper and locust control in special environments by the FAO Desert Locust Pesticide Referee Group.

Applications should conform to regulations concerning pesticide application.

Appendix

I. Germination Test

This test needs to be carried out in a laboratory. Fungal spores are living organisms. The viability of the spores (proportion of live spores) will diminish with time according to the storage conditions. It is therefore essential to know whether the percentage of viable spores has diminished, so the concentration of spores used can be increased accordingly. However, if the percentage is lower than approximately 70%, the spores should not be used.

Use antibiotic agar (Molisch's or Sabouraud dextrose agar) in small Petri dishes.

Suspend a very small quantity (tip of a spatula) of spores in kerosene.

Spread 1/10th ml of the spore suspension on the agar, put the lid on the Petri dish and spread the suspension by agitating. Use the microscope to check that the spores are well distributed at an appropriate concentration. If not, either dilute the suspension further or add more spores.

Incubate at 25°C.

24 hours later use a microscope to count germinating and non germinating spores. Record the time exactly. Any budding spore is considered as germinated.

Count a total of at least 200-300 (germinating and non germinating) spores by moving the field of view of the microscope so as to cover a large area of the Petri dish.

Calculate the percentage of germination as follows:

$$[a/(a+b)] \times 100 = \% \text{ germination}$$

where a = number of germinating and b = number of non-germinating spores

Further information and feedback

More detailed technical bulletins and published information are available from IIBC, IITA, GTZ or CILSS, or from LUBILOSA staff directly.

LUBILOSA welcomes any information, trial results, comments and suggestions from users of Green Muscle®.

II. Glossary/Acronyms

AGRHYMET	AGRonomie, HYdrologie, MÉTeorologie
CILSS	Comité Interetats pour la Lutte contre la Secheresse
Conidia	Non-sexual spores
DFPV	Département de Formation en Protection des Végétaux
FAO	Food and Agriculture Organisation (of the United Nations)
GTZ	Deutsche Gesellschaft für Technische Zusammenarbeit
IIBC CAB	International Institute of Biological Control
INSAH	Institut du Sahel
IITA	International Institute of Tropical Agriculture
Isolate	Fungus taken from one particular place
LUBILOSA	LUTte BIologique contre les LOcustes et SAuteriaux
OAU	Organisation of African Unity
OF	Oil flowable concentrate
Spores	Resting and reproductive stage of a fungus
Strain	Strains are isolates of an microorganism that are recognisable distinct
SU	Oil suspension
TC	Technical concentrate (dry spore powder)
ULV	Ultra-Low Volume

III. LUBILOSA *Adresses*

Biological Control Products SA (Pty) Ltd. (BCP)

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Fax: (031) 700 1338
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Contact Dr. Di Neethling

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For further information, visit the LUBILOSA web site at:

<http://www.cgiar.org/iita/research/lubilosa/index.htm>

Reports and other information can be downloaded from the site, or requested from IITA or CABI.