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Protocol for Cross-species characterization in the reproduction of *Spodoptera sunia* (Lepidoptera: Noctuidae) V.2

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Carlos Ivan Real Baca¹, Carlos Alberto Zuniga Gonzalez²

¹National Autonomous University of Nicaragua, Leon. Nicaragua. Noctuid Insect Breeding Laboratory (CIRCB), UNAN-León Agricultural Campus..;

²National Autonomous University of Nicaragua, Leon. Bioeconomy and Climate Change Research Center



C A Zuniga-Gonzalez

National Autonomous University of Nicaragua, Leon. Bioeconom...



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We use this protocol and it's working

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Abstract

The Protocol describes the process for Cross-species Characterization in the reproduction of *Spodoptera Sunia*(Lepidoptera: Noctuidae). With the characterization it was possible to evaluate the reproductive biological parameters of the species *Spodoptera sunia*.

Image Attribution

Noctuid Insect Breeding Laboratory (CIRCB), UNAN-León Agricultural Campus, coordinates (12.11743, -86.23600).

Guidelines

The protocol Lab is a process for the reproduction of *Spodoptera siuna* in Lab conditions.



Materials

Materials

- 4 and 16 ounce poly cups
- Oviposition cages lined with paper
- Cotton pacifier with 10% diluted honey
- 3% formalin
- 5 pound bags
- 16 ounce poly cups
- 4 ounce poly cups
- Soya diet
- Camel hair brush
- Westover Scientific stereoscope
- Denver Instrument Co Denver Analytical Balance, Cool, Serial No 3570 in Mg
- Plastic cages (10 ltr buckets)

ARTIFICIAL SOYA¹ DIET

	Ingredients	Quantity
	Water	1,660 ml
	soy protein	120 gr.
	red bean protein	275 gr.
	wheat germ	160 gr.
	Yeast	125 gr.
	agar	41 gr.
	methylparaben	10 gr.
	Ascorbic acid	15 gr.
	Vitamins and minerals	2 cdas
	sorbic acid	8 gr.
	belate	2 gr.
	Tetracycline	1 capsule

¹ **Scientific Name:** Glycine max**Common Name:** Soya, soya. **Family:** Fabaceae**Synonyms:** Dolichos soja

Before start

Field tracking

1 Stage 1: Field tracking

The search for *Spodoptera sunia* was carried out in an onion plantation in Sébaco (12.87845, -86.09147), it was inspected in the upper and lower part of the leaves, larvae of different instars were collected, later they were placed in polyethylene cups of 4 and 16 ounces with the leaves and bulbs for food, and were quarantined in the laboratory for up to three generations.

2 Stage 2: Laboratory phase

The assembly of the bioassays was carried out in 2 phases (Adults and larval length). The treatments were: T1: *Spodoptera sunia* species brought from the field, T2: *Spodoptera sunia* species from the Noctuid Insect Breeding Laboratory (CIRCB), T3: *Spodoptera sunia* species Crossing (field-laboratory) established under controlled conditions at a temperature of 27°C and relative humidity of 70-80%.

2.1 Adult Phase

Once the adults had emerged, they were placed in oviposition cages lined with recycled paper with a cotton pacifier with 10% diluted honey and another water pacifier for feeding. This procedure was carried out during the days of the adult's life, counting the mass of oviposited eggs, and disinfected with 3 % formalin for 15 minutes, then they were rinsed with water and dried to be placed in 5-pound bags marked with the date of laying and date of hatching.

Once the eggs hatched, 40 to 50 larvae were placed in 16-ounce polyethylene cups, adding soybean diet to feed them, then the newborn larvae were transferred with a camel hair brush and labeled with their date of laying, date of hatching, and individual transfer date.

2.2 Larval stage length

When the larvae reached their third instar, they were individually transferred into 4-ounce polyethylene cups. Once they pupated, they were sexed with the help of a sexing identification guide (Rizo et al., 1994), taking into account the morphology externa of the abdominal end of the pupa, with the aid of a Westover Scientific stereoscope. The pupae were weighed on a Denver Instrument Co Denver Analytical Balance, Cool, and Serial No 3570 in Mg.

Adults: in the test, plastic cages (10 ltr buckets) were used, lining with recycled paper and adding a 10% honey sucker on the surface and another water sucker for their feeding, 13 females and 13 males were deposited in each of the field, laboratory and cross treatments, changing the paper lining daily.

The postures were collected and disinfected with 3% formalin for 15 minutes, then they were placed in 5-pound bags and observed daily to be transferred into 16-ounce polyethylene cups with the help of a camel brush, depositing approximately 50 larvae.

3 Treatments

Treatment 1: *Spodoptera sunia* species brought from the field

Treatment 2: Species of *Spodoptera sunia* from the Breeding Laboratory

Treatment 3: *Spodoptera sunia* species Crossing (field-laboratory)

120 larvae per treatment were selected, 40 in each repetition for a total of 360 larvae in total.

A six-day-old larva was deposited per four-ounce cup with a 2-cm square piece of diet, with the help of a tweezers the larvae were placed on the millimeter sheet to measure the length of each larva, and they were weighed daily. on a Denver Instrument Co Serial No 3570 Analytical Balance.

Variables to evaluate

-Larvae length: Each larva was placed on a millimeter sheet and the length measured in mm was observed.

-Hatching percentage: The number of eggs that hatched was counted visually.

- Weight of larvae: Larvae were weighed on a Denver Instrument Co Denver Analytical Balance, Cool, Serial No 3570.

- Weight of pupae: The pupae were weighed on a Denver Instrument Co Denver Analytical Balance, Cool, Serial No 3570.

- Sex of pupae: The abdominal part of the pupae was observed with the help of a Westover Scientific stereoscope. (Rizo, C., et al. 1994)

-Life cycle: The days it took in each of the larval stages were counted.