# ENTOMOLOGICAL PROCEDURES

**Standard operating procedure on collection of insects**

**1. Title:** Standard operation procedure on collection of insects.

**2. Scope:** The method is applicable to all insects.

**3. Purpose:** The purpose of this procedure is to collect insect pests for identification and insect reference collection and confirming the pest problem identified and reported is of any economic importance.

**4. Terms, Acronyms and Definitions:**

1. Kahle’s solution is used for fixing larvae. The composition of the Kahle’s solution includes:
2. Distilled water - 1500 mls
3. Rectified spirit (96 % ethanol) - 700 mls
4. 40% formaldehyde solution - 300 mls
5. Glacial acetic acid -100 mls
6. Fixing: killing an insect using reagent that does not distort morphology characteristics of the insect such asKahle’s solution, ethyl acetate and chloroform

**5. Apparatus and Materials:**

1. Pencil
2. Field note book
3. Field data sheet
4. Collecting tubes (with and without ethanol/kahle’s solution)
5. Kilner jars
6. Killing jar (with a small amount of ethyl acetate)
7. A spirater (pooter)
8. Compass
9. GPS
10. Camel hair brush
11. Knife
12. Cooler box
13. Disecting Kit
14. Field spring board
15. Panga
16. Forceps (Straight, fine)
17. Labels (for both insects and plants)
18. Secateurs/ Pole prunner
19. First aid Kit
20. Pair of scissors
21. Nets
22. Magnifying pocket lens
23. Traps
24. Beating tray
25. Mounting/ setting board
26. paint brush
27. Binocular
28. Leaf presser
29. Mallet
30. Chisal
31. Whistle
32. Hatchet
33. Jembe and spade
34. Ladder
35. Prunning saw
36. Pinning stage

# 6. Reagent and chemicals:

1. Ethyl acetate
2. Ethanol
3. Kahle’s solution
4. Paint

**7. Methods of Insect Collection**

Several methods are used in insect collection depending on type of insect to be collected. These include:picking, beating, use of sweeping net, malaise trap, sticky trap, pitfall trap and use of natural baits.

# 7.1Picking Method

In picking method one can use a forcep, camel hairbrush, or a pooter (aspirator) to pick insects.

# 7.2Beating Method

A branch is sharply hit with a stick and dislodged insects are collected or counted on a beating tray which is a durable cloth stretched on a one meter frame.

# 7.3 Sweeping Net Method

A net usually made from durable material of small gauge with lightweight frame and handle is used to catch flying insects by sweeping motion in the targeted area.

# 7.4 Malaise trap Method

Malaise trap method is based on the principle that most insects fly or climb upward when confronted with a barrier and also move from a dark to a lighter area. Weak flyers or insects, that drop when they meet an obstruction, are seldom trapped in a Malaise trap. However, Malaise traps are especially effective in collecting Diptera, Hymenoptera and Lepidoptera, but less useful in collecting Coleoptera, Heteroptera and Homoptera. Attractants can be used to increase the rate of capture for specific insects.

# 7.5Sticky Trap Methods

The sticky trap consists of a piece of glass, plastic or wire net suspended from a tree or fixed to a stake. The sticky material coating the object (usually grease or a non-drying resin) such as ‘tangle foot’ (gum) prevents any insect which lands on the trap to free itself.

# 7.6 Pitfall Trap

Round/cylindrical cups with detergent solution or preservative are buried in an upward position on the groundto trap crawling insects. The lip of the round/ cylindrical cups are level with the ground surface. Insects reaching the lip of the beaker slip and fall and are unable to climb back out. The detergent lowers the surface tension hence increasing the number of captured insects.

Pitfalls can be covered to help prevent excessive rain from overflowing the cup, they can have guide vanes that may help guide organisms into the cup, and they may be baited to capture more specific types of insects.

# 7.7 Use of natural baits

 Using honey, insect pheromones, sugar solution to trap insects.

**8.0 References**

1. Entomology in-service training skills
2. J.E.H. Martin (1977) – The insects and arachnids of Canada Part 1 – Collecting, preserving insects, mites, and spiders
3. Annette K. Walker and Trevor K. Crosby (1988). The preparation and curation of insects (Entomology Division, Department of Scientific and Industrial Research, Auckland, New Zealand)

**Standard operating procedure on rearing of insects**

**1. Title:** Standard operation procedure on rearing of insects

**2. Scope:** The method is applicable to all insects

**3. Purpose:** The purpose is rearing insects from immature stages to adult such as eggs, larva pupae and adult. They are reared up to the adult stage for: identification purposes; emergence of biological control for release into the infested field; and incorporation into insect reference collection.

**4. Terms, Acronyms and Definitions:**

1. **Fixing**: killing an insect using reagent that does not distort morphology characteristics of the insect such as Kahle’s solution, ethyl acetate and chloroform.

**5. Apparatus and Materials:**

1. Rearing cages
2. Incubators
3. Kilner jars
4. Clear/ transparent polyethylene containers (perspex)
5. Food trays
6. Host plants

**6. Reagents and chemicals**

1. Honey
2. Water

**7. Methodology**

7.1 Collect infested twigs (as outlined the collection standard operating procedure) that have eggs or nymphs.

7.2 Transport the infested sample materials to the laboratory using clear/ transparent polythene containers.

7.3 Screen the infested sample materials for natural enemies of the target pest.

7.4 Rear the infested samples using the materials listed in section 5 and 6 above.

7.5 Observe the rearing process for the days for emergence depending on the life cycles of the target insect and record in rearing data sheet R1 and R2 shown below.

7.6 Identify the emergence of the target insect

**Insect rearing data sheet R1**

Date collected;……………………………. Collection number:………………………

Locality:…………………………………………..……………………………………………..

Tentative identification:……………………………. …………………………………………..

Indentifiedby:…………………………….. Date:……………………………………

Host:……………………………………………………….……………………………………..

Remarks:……………………………… ………………………………………………………..

NB: a few specimens of eggs, larvae (or nymphs) and pupae should be preserved if available

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Date** | **Type of food** | **Insect stage** **Living Nos** | **Dead** | **Parasite** | **Remarks** |
|  |  | E | L | N | PP | A |  | ? | C | M | P |  |  |
|  |  |  |  |  |  | ♀ | ♂ |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

E= eggs, L=Larvae, N= nymphs, PP= Pupae, A= Adults, ?= Cause of death unknown;

C=accidentally crushed; M=missing; P= killed by parasites; ♀= Female; ♂= male

Recorder’s name:…………………………

Recorder’s signature:………………………….

Field data sheet folio No……………..

Rearing data sheet (R2) Folio No……………………

**Insect rearing data sheet R2**

Registration:…………………

Insect:…………………………….:………………………

Family :…………………………………………..

Order :……………………………..

Date collected:……………………………..…………………………………

Insect activity: (e.g. Defoliation, Debarking, Boring, Sap-sucking, Resting, on flight, parasite, predator and others)………………………………………………………………………………… ………………………………………………………………………………………………………

Host:………………………………………….

Locality:…………………………………..

Altitude:…………………………………….

Detailed rearing history

Rearing data (to include whole story of the insect since rearing started and associated parasites, predators, viruses, bacteria and fungi. The data should come from sheet R1 (above) plus your feelings about the insect. If below space is not enough please turn over the sheet and continue.

……………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………….……………………………………………..

Recorder’s name:…………………………………………………………………….…………….

Recorder’s signature:…………………………. …………………………………………………...

Rearing data sheet (R1) Folio No.……………..………………………………………….

Field data sheet Folio no..……………………………………………………………….

Typed sheet to file 92……………………………………………………………………….

Update record of file 92 cards:

Host plant cards……………………………………………………………………………...

Host insect cards………………………………….………………………………………….

Insect destination:…………………………………………………………………………….

**8. References**

1. Entomology in-service training skills
2. J.E.H. Martin (1977) – The insects and arachnids of Canada Part 1 – Collecting, preserving insects, mites, and spiders
3. Annette K. Walker and Trevor K. Crosby (1988). The preparation and curation of insects (Entomology Division, Department of Scientific and Industrial Research, Auckland, New Zealand)

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**Standard operation procedure on identification and storage of insects**

**1. Title:** Standard operation procedure on identification and storage of insects.

**2. Scope:** The method is applicable to all insects.

**3. Purpose:** The purpose is to identify and store insects for future reference, confirm the pest problem identified and reported is of any economic importance and give information on control and management of the pest.

**4. Terms, Acronyms and Definitions:**

1. Fixing: killing an insect using reagent that does not distort morphology characteristics of the insect such as Kahle’s solution, ethyl acetate, chloroform,
2. Kahle’s solution is used for fixing larvae. The composition of the Kahle’s solution includes:
3. Distilled water-1500 mls
4. Rectified spirit ( 96 % ethanol) - 700 mls
5. 40% formaldehyde solution - 300 mls
6. Glacial acetic acid -100 mls
7. NARL -National Agricultural Research laboratory.
8. IIE- International Institute of Entomology
9. ICIPE-International Centre of Insect Physiology and Ecology

**5. Apparatus and Materials**:

1. Mounting board
2. Stainless pins
3. Vial tubes
4. Forceps
5. Magnifying glass lens
6. Dissecting microscope
7. Carmel hair brush
8. Scissors
9. Surgical blade
10. Kilner jar
11. Pinning stage

**6. Reagents and chemicals**

1. Kahle’s solution
2. Ethyl acetate solution
3. 75% Ethanol
4. Naphthalene balls

**7. Methodology**

7.1 **Killing and preservation of insect larvae**

7.1.1Prepare the Kahle’s solution using the reagents in the defined quantities as below and pour into vial tubes

1. Distilled water - 1500 mls
2. Rectified spirit ( 96 % ethanol) - 700 mls
3. 40% formaldehyde solution - 300 mls
4. Glacial acetic acid -100 mls

7.1.2 Introduce the live larvae into the vials and wait for 10-15 minutes for them to die.

7.1.3 Prepare the 75 % Ethanol by adding 750 mls of 96% Ethanol in 200mls of distilled water.

7.1.4 Transfer the dead larvae to 75% ethanol for preservation. As an alternative to alcoholic preservation, larvae may be freezed, dried or inflated and then mounted on pins

7.1.5 Identify using a microscope the preserved dead larvaebased on morphological characteristics and reference material.Identification is done using reference collection, in consultation with National Museum of Kenya, ICIPE and NARL.Insect identification keys are also used.The insects, which cannot be identified locally, canbe sent to IIE in London for further identification

7.1.6 Label well the identified larvae. The label should have locality, date, collection number, collectors name, host name and insect identification name.

The insects are arranged according to their orders, families, genera and species.

**7.2 Killing and preservation of adult insects**

7.2.1 Introduce the adult insects into a kilner jar, add a few drops of ethyl acetate and leave to standovernight for the insects to die.

7.2.2 Remove the dead insects from the kilner jar and spread on setting boards with pins.

7.2.3After a fortnight, the adults are well dry and may be removed and labeled. The label should contain locality, date, collection number, collectors name, host name and insect identification name.

7.2.4 Identify using a microscope the well labeled adult insect based on morphological characteristics and reference material.Insect identification keys may also be used. Identification is done using reference collection, in consultation with National Museum of Kenya, ICIPE and NARL.

The insects, which cannot be identified locally, can be sent to the IIE in London for identification.

7.2.5 After mounting transfer the insects into insect storage boxes/ drawers containing naphthalene ballswhich are replaced after every 6 months to protect the insects from being damaged by harmful organisms like mites, cockroaches and ants.

***N.B.*** Never pin or mount the insects when they are alive. The main purpose of mounting an insect is to help the taxonomist to see various parts of an insect without damage and without much strain. When killing the insects using ethyl acetate, make sure that the lid of the jar is well tightened.

7.3 Report writing

After identification of the target pest, a report is compiled and consists of

* Title
* Staff involved in field work including driver
* Objectives of the visit
* Summary e.g. of insect damage
* Introduction
* Areas visited
* Work done and problems encountered
* Conclusion and acknowledge help from foresters or Zonal Forest managers

**8. References**

1. Entomology in-service training skills
2. J.E.H. Martin (1977) – The insects and arachnids of Canada Part 1 – Collecting, preserving insects, mites, and spiders
3. Annette K. Walker and Trevor K. Crosby (1988). The preparation and curation of insects (Entomology Division, Department of Scientific and Industrial Research, Auckland, New Zealand)
4. Insect identification manuals by International Institute of Entomology (I.I.E)

**Standard operating procedure on sampling /monitoring pest outbreak.**

**1. Title:** Standard operating procedure for pest sampling/monitoring pest outbreak

**2. Scope:** this method is applicable to all insects but generally for exotic /invasive insect pests

**3. Purpose:** The purpose of this procedure is to:

1. Assess the extent of damage on tree host.
2. Estimate the population size of the insect pest so that intervention can be taken in case of pest outbreak.
3. Find out the incidence and presence of natural enemies.
4. Examine how the population of the insect is changing through time/ year.
5. Establish impact and success of released/ introduced natural enemies

**4. Terms, Acronyms and Definitions:**

1. PSP – Permanent sample plot
2. Sample – A representative of a given population
3. Sample unit – Is what is actually counted or scored
4. IIBC – International Institute of Biological Control
5. KEFRI – Kenya Forestry Research Institute
6. FRIM – Forestry Research Institute of Malawi
7. Siviculture - is the practice of controlling the establishment, growth, composition, health, and quality of forests to meet diverse needs and values

**5. Field equipment:**

1. Collecting polythene bags
2. Ladder
3. Secateurs/ pole pruner
4. Data logger
5. Compass
6. GPS
7. Camel hairbrush
8. Hand lens
9. Tally counter
10. Beating tray
11. Ribbons
12. Brush and Paint – white or yellow
13. Random numbers
14. Ruler/ measuring stick
15. Recording data sheets
16. Pencil, rubber and clipboard

**6. Laboratory Equipment**

1. Microscope
2. Glass tubes
3. Rearing boxes
4. Specimen box

**7. Method**

* 1. Establish a PSP taking into consideration tree survival in a plantation, age of trees (less than 10 years old is preferable), silvicultural practices and plot accessibility throughout all seasons.
	2. Generate random number to conduct random sampling. Random number tables available in books of statistical tables can be used or they can also be easily generated and printed from a computer.
	3. Establish a plot size of preferably 20 rows by 20 trees or 30 rows by 30 trees by painting the trees using yellow paint on trunk after every 5th tree.
	4. Sample 100 trees as a sample size and a branch per tree as a sample unit. First select a row at random and then select a tree within the row at random. A branch is also selected from the tree at random from the lower five branches. Then cross the number off in the table to avoid repetition. Larger sample size gives more accurate estimates of population.
	5. Damage category can be recorded as follows :
1. Category 1: 0-10% of canopy brown
2. Category 2: 11-25% of canopy brown
3. Category 3: 26- 60% of canopy brown
4. Category 4: 61- 100% of canopy brown or
5. None - No browning of the foliage
6. Low- Up to one third of the foliage showing browning
7. Medium- One to two thirds of the foliage showing browning
8. High- Over two thirds of the foliage showing browning
	1. Sampling frequency should be monthly to determine how population is changing over time.
	2. Record data clearly in a way that can be understood by someone other than the recorder.
	3. Collect and record meteorological data from nearest recording station
	4. Report writing

After collection of data and data analysis, a report is compiled and consists of:

* Introduction
* Objective of the field work
* Field activities undertaken
* Field observations
* Results
* Discussions
* Conclusion
* Recommendations if any

Copies of report should be submitted to relevant officers/ stakeholders.

**8.References**

1. Day R, S. Atuahene, M. Gichora, E. Mutitu and D. Chacha (November 1993) Biological Control of Forest Aphids in Africa – Sampling Cypress Aphids. Technical Bulletin Series No. 2 IIBC, KEFRI, and FRIM
2. Mutitu K.E (1998) Setting up of permanent Sample plots and Monthly Data Collection. KEFRI IPM Technician In-service Training Skills