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Entomological Technique as Referring to the Life History of Insects

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To the Graduate Council:

I am submitting herewith a thesis written by Andrew Jefferson Wheeler entitled "Entomological Technique as Referring to the Life History of Insects." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agriculture and Extension Education.

, Major Professor

We have read this thesis and recommend its acceptance:

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Accepted for the Council: Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

UNIVERSITY OF TENNESSEE

Upon the request of the Committee on Graduate Study the undersigned
have examined a thesis entitled
Entomological Technique as referring to
the Life History of Insects
presented by andrew J. Wheeler
candidate for the degree of Master of Science in
Agriculture, and hereby certify that it is worthy of
acceptance.

Barton C. V. Jessler Examiners.

THESIS

ENTOMOLOGICAL TECHNIQUE AS REFERRING TO THE LIFE HISTORY OF INSECTS

A Part of the Requirement for the Degree of
Master of Science in Agriculture

UNIVERSITY OF TENNESSEE
KNOXVILLE, TENNESSEE

ANDREW JEFFERSON WHEELER

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INTRODUCTION

According to the statement of Kellogg in American Insects, man's real competitors are not the larger creatures that inhabit the Earth with us. These are relatively few in number and easily controlled: but the tiny creatures - the insects and insect-like creatures are the ones that present a real problem to us. They conquor us many times because they are so prolific and so numerous and because they are so small they often go unnoticed. Our Nation has realized the problem and has appropriated millions of dollars annually for the control of insects. This expenditure, however, is not sufficient and the general public should have more knowledge of insects as well as the other branches of nature study. To a better understanding of insect life and to carry on a more efficient warfare against our insect pests, more trained instructors are needed in our schools, colleges, and universities. Much research and writing has been done in entomology, but there is need of some works of compilation or condensation of large volumes into workable form so that the teacher and student may grasp it quickly and easily. In the following pages I have collected the best information available upon the subjects treated and put it into readable form. It is, however, a mere beginning, and it is presented with the hope that others will carry this work to completion.

A CLASSIFICATION OF INSECTS ACCORDING TO ENVIRONMENT

- I. Geophilous Series (ground dwelling insects)
 - Subterranean (below the surface of the ground)
 - Campestrian (in open fields) 1.
 - Orthoptera (1) Gryllidae Mole cricket Myrmecophila
 - b. Hemiptera

(1) Cynidae - burrowing bugs

- (2) Aphidae root-feeding aphids
- (3) Phylloxera root-feeding phylloxerids
- Lepidoptera C. (1) Acrolophidae
- Diptera d.

- (1) Tipulidae crane flies (2) Asilidae robber flies
- (3) Anthomylidae cabbage root maggot onion maggot

(4) Empidae - dance flies

- (5) Bibionidae march flies
- (6) Therevidae stiletto flies
- (7) Dolichopidae long legged flies
- (8) Cocnomyiidae
- e. Hymenoptera
- (1) Formicidae Harvesting ants Thief ants Mound-building ants Blood-red slave-maker ants Shining Amazon ants Corn field ants
- 2. Sylvan (in woodlands)
 - Orthoptera a. (1) Gryllidae Myrmecophila

- b. Hemiptera(1) Cicadidae jar flies
- c. Hymenoptera
 (1) Formicidae ants
- 3. Arenocolous (in dry sand)
 - a. Neuroptera
 (1) Myremeonidae
 Ant lions
 - b. Coleoptera
 (1) Byrrhidae
 Pill beetles
 - c. Hymenoptera
 (1) Formicidae
 (2) Vespidae burrowing wasps
- 4. Hygrophila (in wet places)
 - a. Collembola spring tails
 (1) Podura aquatica
 - b. Coleoptera
 - (1) Cicindelidae tiger beetles
 - (2) Carabidae ground beetles
 Bembidion carinula
 - (3) Staphylinidae rove beetles
 - (4) Histeridae carrion beetles
 - (5) Dascyllidae soft bodied
 - plant beetles (6) Heteroceridae wariegated
 - mud-loving beetles
 (7) Anthicidae ant-like flower
 beetles
- B. Sübsaxean (under stones)
 - 1. Thysanura bristle tails
 - 2. Collembola spring tails
 - 3. Isoptera termites

- 4. Orthoptera
 - a. Gryllidae hiding crickets
- 5. Hemiptera.
 - a. Cydnia burrowing bugs
- 6. Coleoptera
 - a. Carabidae ground beetles (during day)
 - b. Tenebrionidae darkling beetles
 c. Pselophidae ant loving beetles
- C. Humicolous (dwelling in leaf mold)
 - 1. Diptera
 a. Tipulidae snow flies
- D. Terranean (dwelling on the surface of the ground)
 - 1. Saxicolous (on rocks)
 - a. Orthoptera
 (1) Acrydiidae
 Ciscotettix verruculatus
 Sparagemon saxatile
 Trimerotropis saxatilis
 - 2. Arenicolous (on dry sand)
 - a. Orthoptera
 (1) Acrydiidae
 Trimerotropis maritima
 Trimerotropis eitrina
 - (2) Acrididae short-horned grasshoppers

 Melanoplus agustipennis The
 narrow winged locust

 Melanoplus bivittatus The
 yellow-striped locust
 Dissosteira carolina The
 dusty moadside grasshopper
 Campula pellucida The clearwinged locust

b. Coleoptera

(1) Cicindelidae - tiger beetles

(2) Carabidae - ground beetles

(3) Tenebrionidae - darkling beetles

c. Hemiptera

(1) Phymatidae - ambush bug

d. Diptera

(1) Asilidae - robber flies

- 3. Agrarian (on normal soil)
 - a. Orthoptera
 (1) Locustidae short-horned grasshoppers
 - b. Coleoptera

(1) Cicindelidae - tiger beetles

- (2) Carabidae ground beetles
- c. Diptera

(1) Asilidae - robber flies

- 4. Hygrophilpus (on mud flats)
 - a. Otehoptera

(1) Acrydiidae - grouse locusts
Paratettix cucullatus
Tettigidea laternalis
Tettix granulatum

(2) Glattidae - roaches

b. Hemiptera

(1) Nepidae - water scorpions

- (2) Gelastoconidae toad bugs
- (3) Saldidae shore bugs
- (4) Naeogeidae
- c. Hymenoptera
 (1) Formicidae guest ants
- II. Hygrophilous Series Water loving insects
 - A. Hygrophile (species that live about water and wet places)

1. Hemiptera

- a. Ochteridae
- b. Gelastocoridae toad bugs
- c. Belastomatidae electric light bugs
- d. Saldidae shore bugs
- 2. Odonata dragon gly
- 3. Ephemeridae May flies
- 4. Pecoptera stone fly
- 5. Neuroptera Dobson flies
- 6. Diptera
 - a. Rhagionidae snipe flies
 - b. Dolichopodidae long-legged flies
 - c. Simulidae black flies
 - d. Tipulidae crane flies
 - e. Empidae dance flies
 - f. Ephydridae brine flies
 - g. Chironomodae midges
 - i. Sisyridae spongella flies

B. Aquatic

- 1. Still water division
 - a. Dwelling on the surface of the water
 - (1) Hemiptera
 - (a) Veliidae broad shouldered striders

- (b) Gerridae water striders
- (2) Coleoptera
 - (a) Gyrinidae whirligig beetles
- b. Plankton free-swimming
 - (1) Hemiptera
 - (a) Veliidae broad shouldered water stride
 - (b) Notonectidae backswimmers
 - (c) Corixidae water boatmen
 - (2) Neuroptera
 - (a) Sisyridae Spongilla flies
 - (3) Coleoptera
 - (a) Dytiscidae predacious diving beetles
 - (b) Georyssidae minute mudloving species
 - (4) Diptera
 - (a) Tipulidae crane flies
 - (b) Dixidae Dixa Midges
 - (c) Culcidae mosquitoes
- c. Bottom-resting species
 - (1) Hemiptera
 - (a) Naucoridae creeping water bugs
 - (b) Corixidae water boatmen
 - (c) Nepidae water scorpions

- (d) Hydrometridae water measurers
- (2) Coleoptera
 - (a) Hydrophilidae water scavengers
 - (b) Halipidae crawling water beetles
- (3) Diptera
 - (a) Syrphidae rat tailed larvae
 - (b) Stratiomyiidae soldier flies
 - (c) Empididae dance flies
 - (d) Chironomidae blood worms
- d. Species that burrow in mud under water
 - (1) Odonata dragon flies
 - (2) Ephimeridae may flies
- 2. Running water division
 - (1) Hemiptera half-wing
 - (a) Veliidae surface swimmers
 - (2) Ephemeridae May flies
 - (3) Plecoptera stone flies
 - (4) Neuroptera nerve-wing
 - (a) Sialidae sialids

Alder flies

Dobson flies

- (5) Trichoptera caddice flies
- (6) Coleoptera horny-wings
 (a) Parnidae long-toed

- (b) Dascyllidae softbodied plant beetles
- (c) Dryopidae long toed water beetites
- (d) Psephenidae water pennies

(7) Diptera

- (a) Simulidae black flies
- (b) Blepharoceridae netted winged midges
- (c) Tabanidae horse flies
- (d) Psychodidae moth-like flies
- (e) Thaumaleidae solitary idges
- (f) Rhagionidae snipe flies

III. Phytophilous Series

- A. Hydrophytes Lowland or wetland species
 - 1. Collembola Springtails
 - 2. Orthoptera
 Melanoplus islandicus
 Trimerotropis maritima (seaside grasskopper)
 Stenobothris curtipennis Short-winged
 brown grasshopper
 Orphulella olivacea The smaller spottedwinged locust
 - 3. Lepidoptera Micropterygidae - mandibulate jugates
 - 4. Coleoptera
 Melyridae melyrids
 - 5. Hymenoptera

 Xyelidae Xyelid sawflies

B. Mesophytes - (transitional)

1. Thysanoptera

a. Aeolothripidae - aeolothrips

b. Thripidae - thrips

2. Orthoptera

a. Acrydiidae

Eritettex

Tettix ornatus

Tettix hancocki

Tettix arenosus

Paratettix cucullatus

Naetellix bolivari

b. Acrididae

Sub family oedipodinae

Sub family tryxalinae

Genera

Mermiria

Tryxalis

Syrbula

Orphuella

Stenobothrus

Mecos tethus

Sub family Acridiinae

Genera

Lepysma

Arnilia

Schistocerca

Paroxya

Melanoplus - 25 species

c. Tetigonidae - long horned grasshoppers

d. Gryllidae - crickets

e. Phasmidae - walking sticks

3. Hemiptera

a. Cicadellidae - jassids - leaf hoppers

b. Membracidae - tree hoppers

c. Cercopidae - spittle-bugs

d. Psyllidae - jumping plant lice

e. Aleyrodidae - white flies

f. Coccidae - scale insects

g. Miridae - leaf bugs

h. Tingidae - lace bugs

i. Lyaeidae chinch bugs

j. Neididae - stilt-hugs

- k. Sculetteridae shield-backed-bugs
- 1. Coreidae squash bugs
- m. Pentatomidae harlequin cabbage bugs
- n. Cicadidae jar fly
- o. Aphididae plant lice

4. Lepidoptera

- a. Eriocraniidae the mandibulate jugates
- b. Hepialidae the swifts
- c. Incurvariidae incurvariids case bearers in part
- d. Nepticulidae nepticulids leaf mixers
- e. Cossidae carpenter-moths
- f. Pyromorphidae smoky-moths
- g. Megalopygidae flannel-moths
- h. Eucleidae slug-caterpillar moths
- i. Psychidae bag-worm moths
- 1. Tischeriidae tischeriids leaf-miners in part
- k. Lyonetiidae lyonetids leaf-miners
 - in part
- 1. Gracilariidae grachilariids leafminers in part
- m. Coleophoridae coleophorids leaf miners
- n. Elachistidae elachistids leaf-miners
- o. Heliozelids leaf-miners
- p. Douglasiidae douglasiids leaf-miners
- q. Ethmiidae ethmiids leaf-miners
- r. Stenomidae stenomids borers or casebearers
- s. Gelechiidae gelechiids tineids
- t. Blastobasidae blastobasids tineids in part
- u. Cosmopterygidae cosmopterygids tineids
- v. Yponomeuticae yponomeutids tineids
- w. Scythridae scythridids tineids
- x. Plutellidae plutellids tineids
- y. Glyphipterygidae glyphiterygids tineids
- z. Heliodinidae heliodinids tineids
- a. Aegeriidae clear-wing moths
 b. Oltehreutidae blethreutids tineids
- c. Tortricidae tortricids
- d. Phaloniidae phaloniids
- e. Carposinidae carposinids
- f. Pyralididae pyralids
- g. Pterophoridae plume-moths
- h. Orneodidae many-lume moths
- i. Thyrididae window-winged moths
- j. Hyblaedae hyblaeids
- k. Sphingidae hawk-moths
- 1. Geometridae measuring-worms

m. Diopticae - dioptids

n. Notodonitidae - prominents

o. Lymantriidae - tussock-moths

1. Noctuidae - owlet-moths

q. Agaristidae - foresters r. Pericopidae - pericopida

s. Arctiidae - tiger-moths and footman-moths

t. Euchromiidae - syntoinids

u. Eupterotidae - euterotids

v. Epiplemidae - epiplemids

w. Thyratiridae - thyatirids

x. Drepanidae - drepanids

y. Lacosmidae - lacosomids

z. Citheroniidae - royal-moths

a. Saturniidae - giant-silkworm moths

b. Bombycidae - silkworm-moths

c. Lasiocampidae - tent caterpillar

d. Megathemidae - giant-skippers
 e. Hesperiidae - common skippers

f. Papilionidae - swallow-tail butterflies

g. Přeridae - pierids

h. Nymphalidae - four-footed butterflies

i. Lycaenidae - gossamer-winged butterflies

5. Coleoptera

a. Lampyridae - lighting-beetles

b. Cantharidae - soldier-beetles

c. Meloidae - blister-beetles

d. Rhipiceridae - cedar-beetles

e. Elateridae - click-beetles

f. Erotylidae - clover stalk-borer

g. Throscidae - pseudo click beetles

h. Buptestidae - metallic wood borers
i. Byturidae - raspberry fruit-worm

j. Phalacridae - shining flower beetles

k. Coccinellidae - lady bug family

1. Mexican bean-beetles

1. Lagriidae - lagriid bark beetles

m. Bostrichidae - powder-post beetles

n. Apple twig borer Grape cane borer

n. Scarabaeidae - lamellicorns

Rose bugs

Shining leaf-chafers Japanese beetle Sugar-cane beetle Flower beetles

- o. Lucanidae stag beetles
- p. Cerambycidae long-horned borers
- q. Chrysomellidae leaf beetles
- r. Mylabridae pea weevils
- s. Belidae New York weevils
- t. Curculionidae typical snout-beetles
- u. Platypodidae pin-hole beetles
- v. Scolytidae engraver beetles

6. Diptera

- a. Psycoaidae moth-like flies
- b. Cecidomyiidae gall gnats
- c. Trypetidae trypetids
- d. Nemestrinidae tangle-headed flies
- e. Acroceridae small-headed flies
- f. Bombyliidae bee flies
- g. Syrphidae syrphus flies
- h. Psilidae psilids one example is carrot rust fly
- 1. Chloropidae chloropids frit flies
- j. Geomyzidae geomyxisa pomace flies and allies
- k. Anthomyiidae anthomyiids one example is cabbage root maggot

7. Hymenoptera

- a. Pemphiliidae web-spinning and leaf rolling sawflies
- b. Siricidae horntails
- c. Cephidae stem saw-flies
- d. Cimbicidae cimbicid sawflies
- e. Tenthredinidae typical saw-flies
- f. Argidae argid saw-flies
- g. Cynipi gall-flies or gall-wasps
- h. Chalcididae chalcid-flies

Harmolita - grass and grain joint

worm flies

Euritomids - seed-infesting

eurytomids

Bruchopagus - clover-seed chalcid

Blastophaga - fig insects

· i. Formicidae - ants

Harvesting ants

Fungus growing ants

j. Vespidae - wasps

Hornets

Yellow-jackets

- k. Megachilidae leaf-cutter bees
- 1. Bombidae humble-bees
- m. Apidae honey-bees

C. Xerophytes - (highland series)

1. Orthoptera

a. Acrydiiaae
Hesperotettix pratensis
Paroxya atlantica

Paroxya atlantica
Paroxyafloridiana
Tryxalis brevicornis
Leptysma marginicollis
Gymnoscirtetes puscillus
Aptenopedes sphenarioides
Eotettix pusillus
Eotettix palurtris

b. Acrididae

Melanoplus agustipennis - the narrowwinged locust
Melanplus atlanis - the lesser locust
Melanplus bivittatus - the yellowstriped locust
Dissiteria carolina - the dusty roadside grasshopper
Camnula pellucida - the clear-winged
locust

2. Hemiptera
a. Phymatidae - the ambush bug

3. Lepidoptera

a. Ageriidae - the clear-winged moth

b. Noctuidae - the nightfliers Erebus odora - black witch

c. Pieridae - pierids

Pieris protodice - Southern cabbage

worm

Colias eurytheme - the sulphurs.

COLLECTING INSECTS

Reasons for Collecting

Nothing will teach a student so much about the habits and haunts of insects as the making of a collection. He will learn what insects may be found in or near the water, on high land, on rocks or sandy places, on trees or in woods, in decaying wood, boring in living trees, about carrion, parasites of other creatures, about filthy places and about flowers. He will learn that some insects fly only at night, others only in daytime and still others only at twilight. He will also learn that if he would see the insects emerge he must watch for them in the early morning hours.

The preserved specimens are very valuable in the laboratory because they place in the hands of the student a real specimen many times when a live specimen is not obtainable. They also make possible the arrangement in orders and families which will be a valuable demonstration to the student.

Three types of collections may be made:

- 1. Reference, in which they are classified according to orders, genera and species.
 - 2. Economic, in which they are classified

according to hosts.

3. Illustrative, as the Riker, Denton, and Thymo-plas mounts for lecture work.

Equipment for Collecting

Each of us has the equipment that is most essential, - an inquiring mind, good eyes, and nimble fingers. The following articles are also helpful. It will not be necessary to take them all on one trip unless the collector is collecting specimens from all kinds of places.

The air net is used in capturing active winged insects. It may either be made or purchased, In making a net a piece of number nine wire may be used forming it into a loop bringing the ends together and inserting them into a stick about three feet long that is re-enforced with a ring at the end next to the loop. The loop should be twelve or fourteen inches in diameter or even larger for capturing dragon flies. It should be made so that the handle can be removed readily and the bag should be made with a hem at the top so that the wire may be slipped through the loop. This allows it to be removed and cleaned or renewed when necessary. It is a good plan also to use a rather durable piece of cloth for making the loop and several inches of the top of the bag. To this band of coarse cloth as cheese-cloth or mosquito netting or bobinet may be sewed. It is best to use

a gray or green color. White frightens insects.

When capturing lepidoptera, we must remember that
their wings and bodies are covered in part or wholly
with scales. The following points should help to secu
secure our specimens without injuring them:

Don't allow moths or butterflies to struggle. Grasp them through the net by the legs or body and stupefy them by placing a drop of chloroform on the head and thorax or pinch them and drop them into a cyanide jar.

Don't grasp them by the wings.

Don't put other insects in jar with them.

The <u>clap</u> net is said to be efficient for collecting lepidoptera, but its use is not general.

The <u>shears net</u> is used by some collectors in Germany and is thought to be of special value for collecting hymenoptera and diptera from flowers.

The <u>sweeping net</u> is used to sweep the various kinds of low vegetation to collect insects that may be on it. It is necessarily made stronger than the air net. The loop should be made larger than that of the air net and the handle shorter, only long enough so that the operator may touch the ground with the hoop without stooping. The bag also must be made of stronger material than that of the air net. A good

grade of muslin is generally used. The specimens collected in this way should be stupefied in the net with chloroform and poured out into a small piece of thin cloth, labelled and placed in a tight container with a little chloroform. They may be sorted as soon as the collector returns from his trip

The water net is used to capture active insects in the water or for seineing turbid water to secure whatever might be there. It must be more durable than the air net. The landing net used by fishermen is very satisfactory as a water net. It is a very durable net.

The Needham rake is a device originated by Needham for collecting aquatic specimens. It consists of a rake which stirs up the material in the bottom of streams on ponds and behind this is a bag or net of coarse material into which sticks, mud, and insects are gathered. This collection is emptied on the bank or in a boat and carefully sorted.

A pipette similar to those used in electric battery stations for testing the specific gravity of the fluid in the battery is a convenient article to have when collecting aquatic insects. The pipette should have a large rubber bulb which draws the water into the glass which should also have a bulb in it large enough so that no water will reach the bulb.

The small pinch bar is very useful in collecting specimens in old logs or under bark or stones.

A spade is a convenient tool to use in securing subterranean forms. A trowel or army trencher may be substituted for the spade if space or help is limited to carry the equipment. Perhaps the trencher is the best all round tool for this purpose.

A strong, sharp knife is something that every naturalist carries in his pocket and scarcely needs to be mentioned as an article of the collecting equipment. It will be needed in removing branches having specimens that the collector wishes to take in the natural state.

A pair of <u>forceps</u> should be included in the kit for handling carrion beetles or various insects that cannot be comfortably handled with the bare hands.

A <u>vial of chloroform</u> is a necessary part of the equipment and is used as previously mentioned in the paragraph on the net.

The <u>killing jar</u> is easily made. The essentials of it are these:

Deadly poison

Air tight

Large mouth

Plainly labelled

One very successful type of jar is the one pound honey jar as used on the Knoxville market. is a deep jar, it has the wide mouth and also has a screw top that tightens on a warter turn. For a jar this size one third to one half an ounce of Sodium cvanide is used. It is first broken up fine with motar abd pestel then spread evenly over the bottom of the jar and covered with a half an inch of plaster of Paris which is shaken down well by tapping a table with the jar. Now cut three circular pieces of blotting paptr that will fit snugly into the jar. Put one in dry. moisten the second and put the third in dry. The one moist piece of blotter will contain sufficient moisture to cause the cyanide to generate gas. The blotting paper keeps the specimens off of the plaster of Paris and absorbs any fluids that might come from the bodies of the insects and the blotter may be changed whenever it becomes soiled and if the bottle becomes weak, the second blotter may be moistened again. There are other ways of making the cyanide har that are not so good. After the cyanide and plaster of Paris have been put into the jar, water may be added to the plaster of Paris until it is well moistened over the top, at least, and this allowed to set and dry with the bottle open for a day. Another way is to place a half inch of sawdust

over the cyanide then mix the plaster of Paris with water and pour it over the sawdust and shake it down until it is level. An entirely different way of making a killing jar is to place the poison in the lid. This plan makes it possible to have several jars for insects with only one dose of poison. When a jar becomes filled with insects, we simply exchange lids. To make a jar this way a short stout vial is charged with cyanide and attached to the lid by a cork which is glued to the lid. In the cork a hole is made to fit the vial and the vial is glued into this hole. Another plan would be to rivet a small metal cup to the lid and sharge it with cyanide.

Use the cyanide jar only for adult, air-breathing insects. Do not place larva in the cyanide jar as it will spoil them. Do not place aquatic insects in the jar as it will not kill them or at least not quickly. Kill them with boiling water. For best results, do not kill dragon flies in the killing jar. Keep them in paper envelopes and the natural colors will be retained in their wings.

There should be two sizes of killing bottles, one small tube for small specimens, and a large one for the medium and large specimens.

Paper envelopes are needed when an extended trip is made and much collecting done. It is necessary to take precautions to preserve the specimens so that they will not be damaged, especially the lepidoptera, and other delicate winged specimens. The simplest and most successful method of caring for these specimens is to place them in the triangular paper envelopes and carry these in a tin box. The tin box is more durable than cardboard and lighter than wood. The paper envelopes should be made up previous to the trip or at least the paper out in the proper size. It might be well to provide three sizes made from paper 3 x 4 inches, or 4 x 5 inches, and 5 x 7 inches. They must be made of newspaper or some other porous paper so that if it is necessary to relax them they may be left in the envelopes. The moisture will penetrate such envelopes. If the paper were glased it would not. When the specimens are placed in the envelopes, fold the wings together over their backs. Cuts showing method of making envelopes are shown at the end of this section.

Numerous containers for insects besides the killing jar and paper envelopes should be provided. These
should be tin boxes and glass bottles for larvae, aquatic
specimens and any others which the collector desires to
take home alive.

A bottle of 5% formaldehyde is good to have in the kit for preserving larvae that may be kept in liquid preservative. As good a plan as any is to place a drop of chloroform on them and drop them into the formaldehyde.

Night Collecting

There are three methods of night collecting:

Attracting with a bright light

Attracting with baits

Hunting with a flashlight

The bright light method may be used in a number of ways. It may be set in the small end of a funnel and made so that the insects strike it and fall into a cyanide jar, or pan of water and oil, or in gasoline.

Another good plan is to place a strong light between two sheets that are stretched over poles. The insects will alight on the sheet and the collector may place a cyanide jar or any other container over them.

Some night flying insects may be captured by the use of baits. one bait is a thick syrup made from brown sugar to which some vinegar or crushed lemon or orange or old bananas with peeling is added. Smear this on trees in the woods after nine o'clock at night and watch with a flashlight. Moths will be found sipping this bait and may be captured by placing a collecting

jar over them.

The collector may learn much about the habits of insects and may capture many by stealing about at night with a flashlight. He will find the nocturnal insects active, flying or running about, and he will find the diurnal insects in their hiding places.

Rearing Cages

Every department of entomology should be provided with a number of rearing cages to be used for experiments and life history work. These may be boxes screened on one side. They may consist of flower pots containing plants over which lantern globes are placed. These have cloth tied over their tops to keep the insects from escaping. Cocoons may be gathered and allowed to hatch and the young insects studied as they grow. The different molts may be noted and the length of time for each. The different types of metomorphosis may be shown. Experiments with insecticides and parasites may be conducted. The number of broods per year may be shown and many other helpful things.

Aquarium

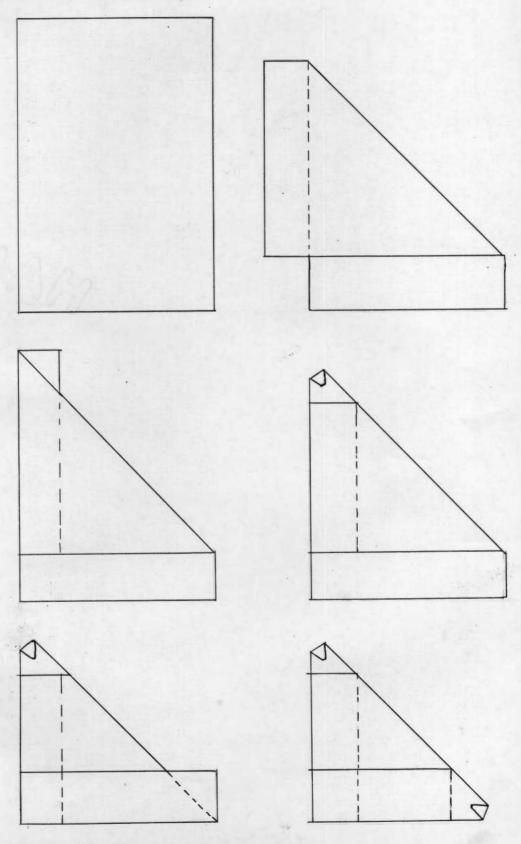
The aquarium is very interesting to students and helps them to study aquatic insects. A large glass

cylinder is very desirable for an aquarium and if we wish, we may put a constant level siphon on it and keep water running through it all the time. There should be some sand and gravel in the bottom of the jar and some water cress and Elodea should be planted in it to keep the water firesh in case there is not a stream running in and out of it all the time. The students may be taught to find water insects and they will bring many to the laboratory.

Terrarium

The terrarium is made of boxes of earth of different types showing different insect habitats as low, moist lands, transitional zones and dry lands. These boxes are arranged side by side and the various types of insects placed in them. The lowland may be placed next the aquarium and the highland farthest from it and the different types of plant and insect societies will be shown in their true relationship from the water to the hilltop.

DRAWINGS ILLUSTRATING THE METHOD OF FOLDING INSECT ENVELOPES



Lutz, Field Book of Insects, pp. 25.

ENTOMOTAXY

Pinning Insects

Most adult specimens are mounted on pins in tightly fitting boxes. These boxes have a layer of cork in the bottom to facilitate pinning. Specially prepared insect pins are used. These are superior to the ordinary pins because they are made of steel and are harder and stiffer. They are longer, sharper, and will not corode. The pin should be pushed through the specimen until about three eights of an inch remains above it. Most insects are pinned through the thorax, but there are some exceptions. The proper way to pin beetles is to thrust the pin through the right wing cover close to the center of the body and about a third of the way back. In grasshoppers the pin is thrust through the thorax just to the right side of the carina. It is essential that the carina be left intact as it is a taxinomic character. Some specimens as the lepidoptera and the odonata are first pinned to a spreading board until dry before placing in a permanent box. This is to give the wings the proper spread. The hind margin of the fore wing should be perpendicular to the body. The anterior margin of the hind wing is brought forward till it lies under the posterior margin

of the front wing. Strips of paper should be used to hold the wings in place and as few pins stuck through the wings as possible.

The Relaxing Jar

The relaxing jar is used to soften specimens that have been kept for some time and are too dry to mount. It has two compartments, one for the specimens and one for water below it. There is a perforated plate between the two compartments. A few drops of phenol is added to the water to keep down molds. The specimens are placed in the relaxing jar in the envelopes and left there about a week or until relaxed enough so that their wings may be spread properly without breaking.

Mounting on Points

Small specimens are glued to points of white card-board or celluloid and these are pinned in boxes as the larger insects. Another plan is to pin small specimens with tiny pins to the point of a triangular piece of cork which is pinned as the other points.

If it is desired to arrange the legs of a specimen, a piece of card-board the proper size may be slipped up on the pin and the legs arranged and pinned upon this. This devise may also be used to support a heavy soft abdomen until it is dry.

Riker, Denton, and Thymo-plas Mounts

Mounted specimens of insects are valuable to the teacher in the class-room and the laboratory and are also valuable as specimens in the museum. For the reference collection, the specimens are usually kept in the all wood boxes. For display as in a museum, a glass topped box is used. There are two types of mounts that are valuable for the teacher, especially. These are the Riker mount and the Denton mount.

The Riker mount is simply a cardboard with a glass or celluloid top. The insects are usually placed on cotton in the lower part of the box and the top is put on and it presses the insects into the cotton enough to hold them in place. This type of mount is durable and endures a great deal of throwing around, hence is good to be use in the laboratory where it is passed around the class for inspection. The mount may show the various stages in the life history of the insect along with a part of the affected plant showing the injury done or the way the eggs are laid. The eggs, larga, and pupa may be placed in vials and placed in the mount along with the male and female adults. Good pictures may be included in the mount showing the various stages of the insect, its work or its control.

Insect parts, as mouth-parts, antennae, or caudal appendages may be mounted on cards and these places in the Riker mount. Such parts may also be glued to the under surface of the glass top of the mount.

The Denton mount consists of two plates of glass held apart by stripes of wood and taped about the edges with Passe Partout. This mount permits the specimen to be studied from both sides. It is not so durable as the Riker mount since it is mostly glass. It is more easily made in the laboratory however, which is an item in its favor. In both the Riker and the Denton mounts it is a good practice to use the photographic label. These are made by taking a picture of large print and reducing it to small size. As many prints may be made as desired and these may be pasted to the lower side of the glass or if a card is used, they may be pasted onto it below the specimen. By this method a very whear label is secured and as small as desired.

Thymo-plas mount is made by combining plasticine, a modeling clay with a strong preservative. To use it take a recently killed insect specimen and place it upon a celluloid slide with legs spread and with wings and antennae in position. For an insect as a fly take a piece of thymo-plas the size of a pea, lay it

on a piece of window-glass and roll it with another piece of glass until it is a long roll a little thicker than the insect's body. Now press it gently with the glass to flatten it a little. Hold it in a loop above the specimen and gauge the size necessary for the specimen. Cut off the surplus ends and lap and press the ends together then place about the insect. Place on this another celluloid slide with its four sides coinciding with those of the lower slide. take the small piece of glass furnished with the outfit and press on the upper slide flattening the thymo-plas and until there is slight pressure on the specimen. This holds it in position. It will help to use a little balsam or glue to hold the insect in place. Lay the specimen aside for 24 hours and if there is no cloud of moisture on the slide, the binder of adhesive paper may be placed around it. If there is moisture, the top slide should be raised for a day. Very soft specimens should be dried for a time before mounting. In cases where thick-bodied specimens are used, a wad of plasticine the thickness of the insect should be placed between the slides at each end to keep the slides from being drawn closer together at the ends than the middle. If the binding does not stick well the edges of slides may be roughened with a flat file. The slides should be cleaned with a moist cloth before used.

This form of mounting has many possibilities.

These slides may be made in any size desired and an innumberable number of specimens may be mounted.

Pairs of insects may be mounted together to show the distinguishing characteristics of each.

All the stages of an insect may be placed on one slide or the insect and the host showing the damage done.

A pupa may be shown inside a cocoon.

Insect mimicry may be shown.

This method is valuable also for mounting delicate specimens as mosquitoes because they may be mounted before they become dry and brittle and liable to be easily broken. When mounted they may be studied with no danger of injuring them.

Specimens may be studied with lens or scope.

They may be used in the classroom and laboratory or may be mailed without being injured.

They may be stored in compact form.

I mounted a number of specimens with thymo-plas and it is a success from the standpoint of preservation and durability. The operations seem awkward at first, but skill may be obtained by practice as with other manipulations.

The chief objection to the process in my mind is

that it does not have a scientific appearance but rather that of child's play.

Another objection would be the difficulty of securing materials from a foreign country.

Permanent Histologic Slides

Legs, wings, antenna, mouth-parts, genitalia, blood, tissues, and many other parts of the insect may be mounted on permanent slides and are very valuable and instructive to students of biology. To the taxonomist they are of great help in classifying speciment, and to the teacher in the laboratory they help to make clear to his classes obscure points that cannot be easily determined with the ordinary lenses.

Inflating Larvae

The object of inflating larvae is to put them in good, permanent, durable condition for external study in the laboratory.

There are two methods of inflating insects. One is with air, the other with paraffin.

The hot air method. Kill the specimen by placing for a few minutes in a bottle with a little ether or chloroform or drop for an instant in boiling water. As soon as it is dead, make in incision in its caudal end

and force the body contents out by rolling with a glass rod. This must be done thoroughly and immediately after death or the specimen will be discolored. The above operation may be done on a sheet of blotting paper or under a spicket of running water. Now insert a pointed glass tube into the incision and seal with celloidin. Attach the glass tube to an air pump with a rubber hose and inflate to its normal size and hold in a drying oven until all moisture is removed. An oven may be improved by placing a pan of sand over a flame and laying a lamp chimney in the sand. The drying may also be done over a hot steam radiator. Specimens inflated with air are very delicate and should be carefully mounted at once. There are two ways of mounting such specimens. They may be mounted on cotton in the Riker mount or they may be mounted by the following method: Twist a piece of small wire about a piece of cork and leave the two ends equal and about as long as the larva. Spread the two ends apart then press together and insert in the incision. Pin in box by pinning through the cork.

The parrafin method. Kill by dropping in boiling water for a second. This is the best method of killing preparatory to inflating with paraffin. It distends the specimen and in case the insect has eversible glands, as Papilio pelyxenes, these are forced out and remain out after the specimen is mounted. Proceed as before to re-

move the body contents. Now melt a small quantity of paraffin which has a melting point of 55 degrees C. If desired, some stain may be added to the melted paraffin. In the case of green larva, Paris green may be added to help to give the specimen when inflated its natural color. Take a syringe, warm it over the flame, and take up some of the melted paraffin with it and fill the larva skin. Now before the paraffin has a chance to cool, take the thumb and finger and force the paraffin toward the head distending the specimen to its normal size and hold it in cold water until the paraffin hardens, then repeat the process until the entire larval skin is filled. When the body content is removed, the skin shrinks to about half its normal size, hence it must be distended and this takes considerable pressure. These specimens may be pinned in boxes, but because of their weight and danger of softening in hot weather, they should rest on the bottom of the box. Another way to store them would be to use a shallow box, place a soft cloth in the bottom of it, lay the specimens in and cover with a soft cloth and pack so that they are tight in the box. This method would not do for specimens having delicate appendages. Specimens prepared in this way are much more durable than those inflated with air and with reasonable care to protect the appendages, they

will serve a long time in the laboratory.

Preserved Specimens in Formalin or Alcohol

It is always desirable to preserve many specimens in alcohol or formalin. For this purpose a uniform vial should be used, the shell vials or the Comstock bent neck vials. These vials are made in different sizes and individual specimens or a few of a kind may be kept in them. puts them in good form for observation. Larvae are usually preserved in this way and some adults also. Insect eggs may be preserved in either alcohol, or they may be mounted on slides in cells filled with glycerin jelly. Pupae may be killed in alcohol or chloroform and preserved in alcohol or formalin or pinned in a box as are the adult insects. Some forms are mounted on a white card, labelled anddropped into a vial of preservative. Small forms or those that are to be used for dissection in the laboratory are best kept in a liquid preservative. Specimens for dissection are killed and kept in chloral hydrate.

Preservation of the Collection

The fumigation of insects specimens. Dried insect specimens must be cared for religiously or they will be destroyed by various insect pest. The chief offender is Anthrenus verbasci one of the Dermestid beetles. The

Dermestids are also called skin beetles. Both the larve and adult feed on dried museum specimens. precaution must be taken to keep specimens shut tightly away from them, but to be sure that they are safe, we must fumigate them once a year or less. The most common material used for this hydrocyanic acid gas. Small collections may be fumigated in any tight box or can, but if the collection is large as in a museum or University, a fumigator should be constructed purposely for this work. This must be built as tightly as possible and arranged so that the cyanide may be dropped in after it is loaded and sealed. Also it should be equipped with a fan so that it may be ventilated after the specimens have been subjected to the gas for a the fumigator sufficient length of time. In loading the boxes containing the specimens should be opened so that the gas may get to the insects readily. When loaded, the jar containing the water and acid should be put in place and the door closed and fastened. The standard formula for this work is as follows for 100 cubic feet of space:

Sulphuric acid 2 oz.

Water 4 oz.

In practice it has been found that this does not always kill one hundred per cent, so to make sure it is well to make the dose double or triple strength. It will do the specimens no harm. The fumigator in the Department of

Entomology here at the University of Tennessee has a cubic content of 33 cubic feet and the formula for 100 cubic feet is used. The regular formula was not always successful, possibly because of the loss of gas through leaks or from lack of penetration. The sodium cyanide is dropped into the acid and water through a small hole which is closed with a cork. The cyanide is tied up in a cloth or bag. The room where the fumigator is must be locked and labelled "poison", "danger" or some other sign to keep people away. The fumigator should be kept closed for twenty four hours and then ventilated thoroughly. When the specimens are taken out they should be boxed up tightly to keep the insects away from them.

Carbon-bisulphide is also used in the form of gas to kill insects. It is a clear, colorless liquid that vaporizes quickly when exposed to the air. Its vapor is 2.63 times heavier that air. This makes is valuable as a fumigant for stored grain. If the container is absolutely air tight, only two pounds for one thousand cubic feet of space are needed, but if there is more or less leakage more should be used. In fumigating insects only a small smount will be needed anyway, and it means considerable loss of time and added expense

if the treatment fails, so it is wise to make the dose large enough so that we will be absolutely sure of success. Then using carbon bisulphide, the same precautions must be taken in regard to looking and posting with the added warning to keep fire and lights away because t is substance is highly inflamable. It will be most deadly when the temperature is above 65 degrees F. as it will volatilize more readily at high temperatures. If the specimen boxes are tight and there is room in them the carbon bisulphide may be simply placed in the box by saturating a bunch of cotton with it and pinning it in the box. The pinned or mounted specimens should be kept dry and in a dark place. Light makes the live insects more beautiful, but after death, its effects are to cause its beautiful colors to fade.

Repellents

Para-dichlorobenzine. As the insects come from the fumigator, or when they are being put away after the treatment with carbon bisulphide, the additional precaution should be taken of placing in each box a teaspoonful of para-dichlorobenzine to act as a repellent. This should be wrapped in some newspaper

and pinned in one corner of the box. This treatment may well be given every three months. Records should be made of the date of the fumigation. No insects should be left around the laboratory or museum to become breeding places for the Dermestids.

Specimen boxes. Insect boxes of various types and makes are on the market. The Schmidt box is the best one. The top and bottom fit very closely together and are made of two pieces glued together so as to prevent warping. The lid is hinged and held shut by brass hooks and eyelets. On the bottom inside is a layer of cork and the box is lined throughout with white paper. The Schmidt box may be obtained in sizes $8\frac{1}{2} \times 13$ inches or 12×15 inches.

The Comstock box is larger, 16 x 19 inches. It has a glass bottom as well as a glass top. This is a weak point because it is difficult to keep the box tight about the glass.

Fire-proof steel cabinets. If the collection is kept in a steel cabinet, it may be protected from destruction from fire. A collection of insects represents a great deal of time and effort as well as good for tune in securing specimens that often t imes are rare so that it is well worth every precaution to

preserve it. A shelf four feet long, ten inches high, and fourteen inches deep will accommodate sixteen

Schmidt boxes and a cabinet with six such shelves would hold 96 boxes. A two door steel cabinet of this size and arrangement would be prized in many a school for its collection of insects.

Arranging the collection. A well classified collection of insects is of great value to the teacher of entomology or biology. It is hardly expected that the amateur will have such a collection because of the immense amount of work and knowledge involved. However, if a beginning is made and the help of students and other interested persons is enlisted, the collection will grow and in time amount to something. The first collections should be economic insects. The teacher will be aided in naming t ese by the various bulletins that may be obtained from the department of entomology of the state or nation to be named. A number of good keys are now available, some simply for the asking. The instructor should secure some of the latter at least. key is a short cut to learning what a specimen is by the elimination of other groups. It contains a series of opposing statements one of which is eliminated at each step. Students of biology or entomology should be taught to use simple keys early in the course, and such

keys should pertain to those insects most commonly collected by the students and should be non-technical. (How Insects Live - Tellhouse, pp 40.) Collections are of little value unless named. A good supply of bulletins on insects should be in the library available to the students to help them to become acquainted with the various insects.

There are seventeen different orders of insects according to the Folsom method. The amateur should have little trouble in placing insects in their proper order and possibly in the family and genus. The adult insects will be placed in the insect boxes or if these are not obtainable, in cigar boxes which will be labelled on the end with the name of the order and family.

The various larvae are preserved in vials and arranged according to order and family by setting the vials in holes bored in thick boards. Larvae are more difficult to classify than adults as the keys have not yet been worked out. These may be determined by rearing them from the eggs of known adults.

A	LIST	OF KEYS FOR T	RE CLASSIFIC	CATION OF	INSECTS
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		Ohio State Uni	iv. Studies	Vol., 2,	No. 2
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2.	The	Butterfly Book	S		. Holland
		Doubleday, Pag	ge & Company	,	
		1903			New York
3.	The	Cicindelidae o	of Kansas		W. Knaus
		Can. Ent., Vol	L. 32, No. 4		
		1900			
4.	Clas	sification of Ch	Culucidae o		Vestiture F. Knab
		Ent. News, Vol	. 18		r • Anab
		1907			
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		Entomologica 1	mericana, N		
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		James T. Hatha	iway		
		1906		New Hav	en, Conn.

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10.	Cole	eoptera of Southern California
		1901
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		1878
12.	The	Collembola of Minnesota Joseph E. Guthrie
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Albany, N. Y.

1918

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		Office of State Printer	
		1923	Hartford, Conn.
22.	Hete	eroptera of Eastern Forth	America
		The Nature Publishing Com	npany
		1926	indianapolis, ind.
23.	The	Hymenoptera of Connecticu	W. E. Britton
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24.	The	Hymenoptera of Colorado -	W. H. Ashmead
		Bul. No. 1, Col. Biologic	eal Assn.
			Washington, D. C.
25.	Clas	ssification of the Ichneum	W. H. Ashmead
		U. S. National Museum, Vo	ol. 23, pp. 1-220
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		1905			Albany,	N. Y.
29.	Synd	optical Keys	to the	rica Mirio	the Normaliae	
		University o	f Calif	Cornia		
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		N. J. Agricu	ltural	College		
				New Bru	unswick,	N. J.
31.	Moso	quitoes of "e	w York	State	E. P	. Felt
		New York Sta	te wase	um		
		1904			Albany,	N. Y.
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					S. B. Fr	3 6 TO LII
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		1926			Berkley	, Cal.
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		Univer	sity	of Cal	iforni	.a				
		1926				Berkle	y, Ca	life	orni	la
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	ŗ	The Na	ture	ublis	hing C	ompan	y			
		1903				India	anapol	is,	Ind	l.
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		1920				India	anapol	is,	Ind	
40.	Paece	optera	of A	merica J. G.	North Needb	of me	xico P. W.	Clas	asse	n
		Thomas	Say	Founda	tion					
		1925				Le	afayet	te,	Ind	

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	1915 Washington, D. C.
46.	Syrphidae of Ohio C. L. Metcalf
	Ohio State university
	1913 Columbus, Ohio
47.	Syrphidae of Maine C. L. Metcalf
	Buls. 253 & a56 Maine Agr. Exp. Station
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48. The Syrphidae of Maine C. L. Metcal	
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19. Syrphidae of Colorado C. R. Jone	
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50. Syrphidae of Ohio C. L. Metcal	
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1882	

GENERAL BOOKS ON ENTOMOLOGY THAT CONTAIN KEYS FOR THE CLASSIFICATION OF INSECTS

1.	An Introduction to Entomology John Henry Comstock
	The Comstock Publishing Company
	1924 Ithaca, N. Y.
2.	American InsectsVernon L. Kellogg
	Henry Holt & Company
	1905 New York, N. Y.
3.	Field Book of Insects Frank E. Lutz
	G. P. Putnam's Sons - Knickerbocker Press
	1921 New York, N. Y.
4.	How Insects Live Walter Housley Wellhouse
	The macmillan Company
	1926 New York, N. Y.
5.	Knowing Insects Through StoriesFloyd Bralliar
	Funk & Wagnalls Company
	1918 New York, N. Y.

THE TECHNIQUE OF PREPARING INSECTS FOR HISTOLOGICAL STUDY

The insect should be brought into the laboratory alive to be of value for sectioning.

Killing.

a. Most specimens may be dropped directly into the fixer which is heated in a water bath to a temperature of 70 degrees C. A good fixer to use is:

Five per cent Chloral Hydrate is also a good killing agent for certain specimens and is excellent to preserve specimens that are to be dissected.

that have a high contractibility because it will leave them in a contracted condition and not fit for study. Boiling water is best for killing these. They should be left in the water until the protoplasm has entirely coagulated and then should be transferred immediately to the fixer. This method is best for eggs and larva because they are impervious to the fixing fluid.

Fixing

Functions of fixing agents

* Suggested by B. C. V. Ressler, Asst. Prof. of Zoology, University of Tennessee, Inoxville, Tennessee

change in form. Fixing in death the form or attitude it had in life.

- 2. <u>Hardening</u> at least enough to enable tissue to offer such mechanical resistance to postmortem change and to the processes of after treatment as not to suffer change of form.
- 3. To render insoluble elements of cells and tissues that would otherwise be more or less dissolved by the liquids employed in the after treatment.
- 4. Producing optical differentiation in structures. This is done by their action of altering in varying degrees, by coagulation, the indices of refraction of the different parts of the cell.

The action of fixing agents

This consists in <u>coagulation</u> and rendering insoluble certain of the constituents of tissues. This is done in two ways.

- l. Without any chemical action being involved as when alcohol is used which simply withdraws the water from the tissues.
- 2. Intering into chemical combinations with certain of the elements of the tissues, forming compounds, sometimes unstable and soluble and sometimes insoluble. These chemical combinations are precipitates when the constituents

of the tisues are liquid or semiliquid.

Characters of fixing agents

- A good fixing agent must:
- 1. Preserve all the elements it is desired to fix
- 2. Give good optical differentiation
- 3. Penetrate the tissue so that it is fixed throughout.

 No single substance fulfills all the requirements

 of a good fixative. All the best fixing agents are mixtures.

As a general rule, to get most complete fixation, fixatives should have an <u>acid reaction</u>. So if they are not naturally acid, 1% to 5% of acetic acid is added. Sometimes, however, they should be neutral or even alkaline.

Practice of fixation

- 1. See that tissues are perfectly living at instant of fixation, otherwise pathological post-mortem states will be fixed.
 - 2. Secure rapid penetration of fixing agent by:
- A. Dividing tissues into smallest portions that can conveniently be employed, or make large openings in tissues or specimens.
- B. Heating fixative to boiling point when tissue is put in.
- 3. Use amount of fixative many times the volume of the objects to be fixed. For weak and slow acting agents, 100 volumes of fixer should be used. For stronger fixers, less may be used.

4. Don't leave specimens in fixative longer than is necessary to obtain desired reaction or they may become too brittle.

Fixing Agents Found Most Useful in Insect Histology

1. Picro-aceto-sublimate
(0. Vom Rath: Anat. Anz. XI, 1895)

1 part cold sat. solution of picric acid in 70% alcohol

l part hot sat. aqueous solution of corrosive sublimate

to 1% glacial acetic acid

Put tissue in for from 12 to 24 hours, remove to 70% alcohol for 24 hours, changing the latter 2 or 3 times.

2. Piero-sulphuric-acid
(Kleinenberg: Quart. Journ. Mic. Sci., Apr. 1879, pp. 208.)

Distilled water 100 parts

Sulphuric acid 2 parts

Picric acid as much as will dissolve

Use same as No. 1.

3. Chromo-nitric acid (Ferenyes' fluid)

10% nitruc acid 4 parts

96% alcohol 3 parts

2% chromic acid 3 parts

Fix 4 to 8 hours and pass into 70% alcohol as above.

4. Picro-formalin
(P. Boulin - Phinomenes Cytologiques Anormaus
Dans L' Histogenese, etc., Naney, 1897, pp 19.)

Picrica cid (saturated aqueous solution) 75 parts
Formol 25 parts
Acetic acid 5 parts
Fix 18 hours, place in 50% alcohol then into 70%.
5. Fleming's Solution (Fleming, Zellsubstanz, Kern Und Zelltherburg, 1882, pp. 381.)
1% chromic acid
2% osmic acid 4 parts
Glacial acetic acid
Reep chromic and osmic acid ready mixed in right pro-
portions and add 5, acetic acid at moment of using.
Fix for from one to several days, wash out in running
water for 24 hours. Use very small pieces and strain with
safranin or haemotoxylin. Fine for cytological work.
6. Gilson's solution (Gilson, in litt. 1895)
Bichloride of mercury (corrosive sublimate) 5 gms.
Nitric Acid (about 80%)4 cc.
Glacial acetic acidl cc.
Alcohol (70%)
Distilled water
Fix 8 to 12 hours, wash in 70% alcohol with four
drops of iodine added. If alcohol bleaches, replace by

fresh which has been similarly iodized.

Washing Out

This is the removal from the tissues of the excess of uncombined fixative. This is necessary to get the tissue to stain properly.

Use proper liquid for washing out. Sometimes water will undo all the work of fixation as is the case if used after picric acid fixatives. Sometimes alcohol causes precipitates that may ruin the preparations. The general rule is as follows:

- l. Objects fixed in alcohol, formol, acetic, pieric, or nitric acid are washed out in alcohol.
- 2. Those fixed with osmic or chromic acid are washed with water.

Use liberal quantities of liquid for washing.

Change it as often as it becomes turbid. Heat hastens the process.

Hardening

This is an after process to give additional hardness.

- 1. Amploy a relative large volume of hardening fluid.
- 2. Begin with weak reagent, increasing strength gradually.

3. Remove objects from the hardening fluid when they have acquired desired consistency, or they will become brittle.

For insects after washing out in 70% alcohol, specimens may be kept indefinitely in 85%. For immediate cutting, harden specimens as follows:

- 1. Put in 95% alcohol for 24 to 48 hours depending on size of specimen and degrees of chitinization.
 - 2. Put in absolute alcohol for 24 to 48 hours. It will then be thoroughly hardened.

Clearing or De-alcoholization

De-alcoholization agents are liquids used to get rid of the alcohol used in hardening. They facilitate the penetration of the paraffin. They must all, therefore, have two chracteristics.

- 1. Most be capable of dispelling oil from the tissue.
- 2. Must be the Solvent of Canada balsam or other resinous media.

Function of clearing agents

To make tissue transparent. This they do by penetrating amongst the highly refracting elements of the tissues, the clearing agents themselves having an index of refraction superior to the tissues to be cleared. Therefore, all clearing agents are liquids of high index of refraction.

The majority of de-alcoholization agents are also liquids of high refraction and therefore serve at the same time for de-alcoholization and clearing, and while the two processes shown above are distinct, yet they are generally performed by one agent at the same time. So practically, de-alcoholization and clearing may be spoken of as the same process.

Clearing agents in order of importance.

- 1. Xylol
- B. Cedar oil
- 3. Clove oil
- 4. Carbolic acid
- 5. Carbolic acid crystals melted .. 40 cc.
- 6 Oil of turpentine 60 cc.
- 6. Creosote

Practice of clearing

Remove specimens from 95% alcohol.

Leave for 12 to 24 hours in a mixture of abs. alcohol and xylol, 50-50.

Then in xylol 12 to 24 hours, or,

In mixture of abs. alcohol and cedar oil 50-50, twenty four hours.

Pure cedar oil 24 hours.

Infiltration

Remove cleared specimens to mixture of xylol and melted paraffin 50-50 and leave in oven three to six hours.

Remove to pure melted paraffin and leave in oven

in numbering

- 60 -

for six to twenty four hours.

For insects in general, use a hard paraffin, except for very delicate work. Use paraffin of high melting point, - 52-55 C.

In summer hard paraffin must be used for the sake of ease in cutting.

mbedding

Tissues may be embedded in either paraffin, or celloidin. Each has its advantage and disadvantage. Celloidin is good for large objects, for brittle objects, or friable objects, or delicate objects that heat would injure. Celloidin is transparent and usually does not need to be removed from the tissues. It is not possible to cut thin sections from celloid n blocks, so it cannot be used for cytological work. It is impractical to cut sections thinner than 10 microns in diameter. The celloidin process is more tedious taking three days while paraffin required only an hour. Paraffin is rapid and allows the cutting of very thin sections but is not successful in specimens much larger than one half inch in diameter. The rule is "use paraffin wherever possible." The cellpidin sections have this advantage; when a slide is broken in the laboratory, by an inexperienced student, the sections being tough is uninjured and may simply be remounted, thus all the former, tedious process of preparing the slide, or the expense of a new slide is saved.

Most specimens are embedded in paper trays or boxes made to fit the specimen. The paper is folded so that a rectangular box is made in such a way that a folded end of paper is left at each end of the box. These may be used formandles and labels.

When ready to embed, the specimen we pour a small quantity of paraffin into the bottom of the box and allow it to harden some and then place the insect and fill the box with melted paraffin, arranging the specimen with a warm needle. The specimens should be cooled quickly and should be left in cool water for ten or fifteen minutes. All specimens must be labelled as soon as embedded. They may be kept indefinitely before sectioning. The label should contain the name of the insect, the character of the specimen, and reagents used in its preparation.

It is sometimes advantageous to embed small specimens in a watch-glass.

Sectioning

This is done with the microtome. There are two types, - the rotary and the sliding. The block containing the specimen is trimmed to within one sixteenth of an inch of the specimen and then it is stuck to the disc of the macrotome by melting some paraffin on the disc with a hot needle then setting the block in the melted paraffin and

melting its edges so that they fuse with the other paraf-

Adjust the microtome to cut the proper thickness which is from five to ten microns.

Make sure that the razor blade is fastened securely.

Sometimes it is impossible to operate the microtome. This may be due to the condition of the atmosphere or to the hardness of the paraffin. This may be overcome by placing an alcohol lamp near the microtome so that warm currents of air are carried over the razor and block. The specimen may be re-embedded in softer paraffin or laid away till the atmosphere is right. The sections should stick together forming ribbons. In order for it to do this, both the top and bottom of the bhock must be parallel with the edge of the knife.

As the ribbons come from the knife the are picked up and carried with a camel's hair brush until the desired length is secured when they are laid out on sheets of paper and may be kept in this form. It is safer however, to mount them as soon as possible.

Section Mounting

The slides must be clean. To clean them, wash in a soap suds in distilled water with a little ammonia added, then dip in weak HCL, wipe surplus off and dip in

waste alcohol and wipe dry. If the slide is clean when breathed upon, the moisture from the breath will condense evenly.

Albumen fixative is generally used to stick the sections to the slides. Place a frop on the slide and apread it with the finger. Arrange the sections on the slide and place enough distilled water around them so that they will flatten out, then dry thoroughly after pouring off surplus water.

Place slide in paraffin oven till paraffin melts and remove till paraffin is hardened again.

Removing the Paraffin

To remove the paraffin, place the slide in a jar of xylol for ten minutes. The xylol dissolves the paraffin.

Staining

There are two types of stains, general and specific. The general stain is one that affects equally all the elements of a specimen. The special stain is selective, affecting only certain elements of the specimen. Certain elements will be highly colored by the special stain while others will be colored less or not at all. The real object of staining is to bring about this differentiation so that the various elements may be studied. Details of structure are made more visible.

Stains may be placed in two classes by their modes of selection.

- 1. Histological selection in which all like tissue elements are prominently stained while the other elements remain colorles or are stained less or not at all or in a different manner. This is known as a specific stain.
- 2. Cytological selection in which the stain seizes one of the constituent elements that go to make up the cytoplasm.

Two kinds of stain exhibit cytological selection:

- (1) Nuclear or chromatic stains which have selective affinity for the substance of the nuclei. It is a great help in finding the outline of cells.
- (2) Plasmatic or plasma stains which affect the elements of the cell other than the chromatin, such as reticolum of cytoplasm, granulas, etc.

3. Dyes

Basic, Acid and Neutral

The coloring matters used in industrial dying or histological staining are almost always salts. There are three kinds of these salts.

- (1) Basic. A compound in which a socalled color base (a molecular group to which the compound owes its color) is combined with a new coloring acid. For example fuchsin is hydrochloride of orsanilin. Here the base is composed of rosalin which furishes the color which is not due to the HCL of the compound.
- (2) Acid. A compound in which a socalled 'color-acid' is combined with a non-coloring base. For example acid fuchsin. This is a soda salt of rosanilin disulphuric acid. Its color properties are due to rosanilin which exists as an acid in the compound, and not to the soda. Also picrate of ammonia is an 'acid color' and its coloring properties are due to the picric acid and not to the ammonia.

Words "acid" or "basic" refer to characters of the color bases and not to those of the salts.

Thus an "acid" dye may have a neutral or alkaline reaction, (picrate of ammonia) and vice versa.

Basic dyes are generally easily soluble in alcohol, less easily in water; the contrary is the case for acid dyes. Stain given by acid dyes is fast to acids and may be intensified by them; basic dyes are worked out by acids, but intensified

by alkalies.

(3) Neutral. Are compounds of a colorbase with a color acid prepared by mixing the aqueous solutions of a basic and an acid dye.

Basic stains are in general chro-

Acid stains are in general plasma stains.

· 4. Mordants

combine directly with tissues stained. These are called substantive stains. Other stains do not combine directly with the tissue to be acted upon, but the tissues must first be charged with some substance called a mordant before it will combine with the coloring matter. So mordants are bodies which have the power of combining on the one hand with the elements of the tissues, and on the other hand with the coloring principle of the stain used, forming with the latter insoluble compounds, called Lokes, which are retained in the tissues. Ferric alum is used as a mordant when staining with Heiderbaums Iron Haematoxylin when doing cytological work.

These are called "adjectice" stains.

5. Stains

There are five groups.

- (1) <u>Carmine</u> stains are used chiefly for staining entire objects or tissues in bulk. Grenacher's alcoholic borax carmine is best.
- (2) Haematoxylin stains are used substantively as weak plasma stains, but combined with a mordant become basophilous, and give powerful nuclear stain, or at some time a nuclear and a selective plasma stain. We list a few of the Haematoxylin stains:
 - (a) Heiderbaum's Iron Haematoxylin (Nuclear)
 - (b) Moyer's Haemalum (Nuclear)
 - (c) Delafield's Hematoxylin (Nuclear)
 - (d) Erlich's Acid Hematoxylin (Nuclear)
 - (3) Nuclear stains with coal-tar dyes.

Most stains obtained from coal-tar dyes fade much in a few months or years.

- (a) Methyl green
-) Progressive
- (b) Bismark brown
- (c) Safranin
-) Regressive
- (d) Gentian violet
- (4) Plasma stains with coal-tar dyes.
 - (a) Acid fuchsin

- (b) Orange g.
- (c) Picric acid
- (d) Eosin
- (5) Methylene Blue (Not methyl blue)
 Used for nervous tissue and blood chiefly.

Preparation of Tissues for Staining

After removing the paraffin place the slide in 95% alcohol for five minutes to remove xylol and then for five minutes each in 85% and 70% alcohol. It is now ready to stain if alcoholic stain is to be used, but if aqueous stain is to be used, such as Delafield's Haematoxylin, place the slide in distilled water for five minutes.

Practice of Staining

Selective staining is done in two ways.

- 1. Progressive staining. Here a coloring agent is used that stains desired elements more quickly than those to be left unstained and the process is stopped when the former are stained just right and the latter only slightly or not at all.
- 2. Regressice staining. By this method all elements are thoroughly stained and the elements that we do not desire to be stained are decolorized. Certain tissues retain the stains more obstinately than others because of

their chemical or physical properties.

Better results are generally obtained by prolonged staining in dilute solution, rather than by a short bath in a strong one.

Staining in toto

has been washed out and put into Grenacher's alcoholic borax-carmine for one or two days. Then place in 70% alcohol, acidulated with 1-1000 parts of HCL for differentiation. When no more coloring is being removed, wash in 70% alcohol and narden in 85%; 95%; and absolute. Clear, embed, cut, mount on slide, remove paraffin and mount in balsam.

Clearing and Mounting

For clearing xylol or cedar wood oil may be used.

Take slides from absolute alcohol and place in clearer
for ten to fifteen minutes. Remove from clearer and quickly drop some thin balsam on sections and place cover glass.

Don't allow the section to dry. Remove bubbles with point
of needle. Use no more balsam than is necessary.

Place slides in slide oven until balsam hardens.

Label slides with

- 1. Name of specimen
- 2. Name of fixing agent
- 3. Name of stain
- 4. Date of mounting

It is possible for the microtomist to work out a scheme similar to the one shown below by which he will be able to put much information onto the slide label without crowding. Different schemes may be devised for different bunches of slides. The key may be printed in quantities and one pasted onto the slide box for reference. The different keys may be printed in different colors so that several varieties may be had for the different types of work. The characters in the various positions may simply be checked to indicate the different steps taken in the process of preparing the specimen.

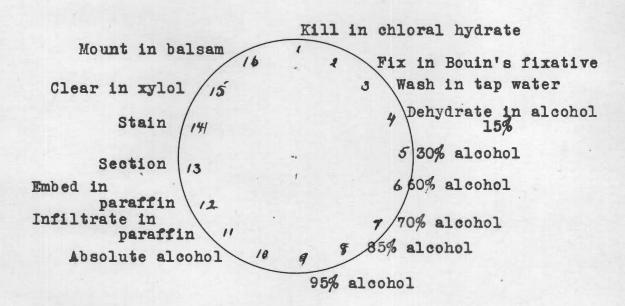
Fixed in Erlicki's

Fixed in Bouin's Stained with haematoxylin

Fixed in Gilson's o Stained with eosin 'Y' Killed in chloral hydrate o Cleared with xylol

Killed in hot water o Mounted in balsam

The idea shown in the chart below is a valuable one for helping students to remember the various steps in the process of preparing permanent slides. It may be made into a permanent wall chart or simply drawn upon the blackboard for them to copy into their note-books.



" Mary

RECORD OF HISTOLOGICAL WORK DONE

The Problem was the preparation of cross and longitudinal sections of several lepidopterous larvae.

The Object was to study the internal structure of the specimens and to learn the technique of slide making for permanent mounts.

Procedure: The specimens were killed in a killing solution made up as follows:

70% alcohol97 parts
Glycerin 3 parts

This solution was heated to 70 degrees on water bath and the specimens were dropped into it and kept in it for 3 minutes. Each larva was then placed in a separate labelled vial of Bouin's fixing solution and kept in this for 24 hours. The larger specimens were cut into two or three lengths so that the solution would penetrate all the tissues. When the fixing was complete, each specimen was tied up in a separate piece of cheese-cloth and placed in a pint jar.

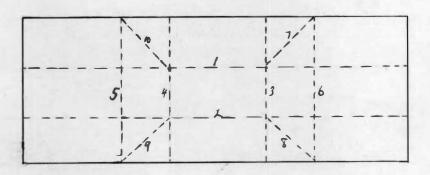
A small slit was made in it and the hose from the spicket was inserted and extended to the bottom of the jar. The tap water was then turned on and allowed to run slowly for 22 hours. From the washing the specimens were run through

^{*} Suggested by B. C. V. Ressler, Asst. Prof. of Zoology, University of Tennessee, Anoxville, Tennessee

various solutiona and embedded in paraffin. This required two days. The first day they were left two hours each in the alcohols 35%, 50%, 70%, and were left over night in 85%. For this work, shell vials one inch in diameter were used and as much liquid as 25 times the volume of the speciwas used. The second day the specimens mens were put through the following:

A supply of paraffin was kept melted in the paraffin oven. The vials containing the 50-50 mixture of paraffin and xylol were kept warm enough to keep the paraffin melted. For infiltration the specimens were placed in melted paraffin in small vessels and kept in the paraffin over for the hour. For embedding, I used a paraffin with a melting point of 56 degrees C and prepared it as follows: I mount; ed a funnel on a stand, placed a filter paper in it and a flask under it. I then took the block of paraffin and held it over the funnel while I played the flame of the gas burner on it. As it melted it dripped into the funnel, through the filter paper and down into the flask. The

process was continued until enough paraffin was melted to embed all the specimens. The next step was to make the paper boxes in which the specimens were to be embedded. A good stiff paper should be used. A good typewriter paper is all right. Fold as shown by the drawing which is actual size.



This size is for small specimens. They may be made larger for large specimens. They should be large enough so that the specimens will not be crowded. The folding will be facilitated if a stick is provided with its end the size and shape of the bottom of the box over which the paper may be folded. The box is next filled about one fourth full of the melted paraffin and this is allowed to cool enouth so that the specimen will not sink to the bottom of the box. The specimen is then put in position and the

paraffin allowed to harden a little more then the box is filled. The specimens were transferred to the boxes with a section lifter and oriented with a hot needle. As soon as the specimen is in its proper position, the box was set in a pan of cold water and left in it for at least fifteen minutes. It was then ready for sectioning. Our microtome is a Bausch & Lomb. The first operation was to take the paper off the paraffin block and trim the block to the right size for mounting on the disc of the microtome. To mount it on the disc, I neated the disc, melted some paraffin on it and set the block in this and with the section-lifter heated, made the attachment solid by melting the sides of the block and running it onto the disc. I then placed the disc in position in the machine and trimmed the block a little more, making sure that the top and bottom edges were parallel. This will help to make the sections stick together in straight ribbons better. I sharpened the razor next and placed it in the holder and moved the stand near to the end of the block. The microtome was then set for cutting sections 10 microns thick and the cutting started. The sections were not saved till the end of the specimen came to the razor, then all the paraffin was cleaned from the machine. Then as the sections came from the microtome. I picked up the end with a camel's hair brush and carried it out straight in front

R

of the machine until it was about ten inches long and with the aid of a pencil ar handle of a probe, removed it from the cutter and laid it on a piece of clean typewriter paper. This process was continued till the entire block was sectioned. Before sectioning more, the sections from this specialen were mounted on slides. The slides had been previously cleaned by washing in water with a few drops of HCL in it and dipped in commercial alcohol and dried with a clean cloth. To mount the sections a small drop of albumen fixative was placed on the slide and pread with the finger. The ribbons were cut and transferred with a scalpel. They were cut in lengths somewhat smaller than the cover glasses to be used because in the process they spread and expand some. After the sections are in place on the slide, float them in water and hold over a flame an instant to warm the paraffin so that it will spread and stick to the glass. If this process is not carefully done the sections will come off the slide in the process of staining. Care must be taken that the paraffin is not melted. As the sections were mounted the slides were placed in the slide boxes and the boxes labelled on the outside so that each specimen was kept separate from the other. With some of the specimens only part of the sections were used, but in most of them serial sections were made, that is, all the sections were mounted in the order they were cut.

In preparation for staining, the necessary slide vials were secured, twenty of them and washed in warm soap water and rinsed in warm clear water. They were thoroughly dried and set in a convenient position on the work table and labelled with the name of the solution that was to be placed in them. The solutions were then put in them. We had previously distilled the absolute alcohol. Delafield's Haematoxylin and Eosin 'Y' stains were used according to the schedule at the close of this section. With this schedule and the watch in front of us, staining was begun. Each vial held five slides and a new set was started every thirty minutes. I kept track of the time by placing under the lid of the vial in front of the slides a slip of paper with the time when the slides should go into this vial. The entire time required to run a set of slides thorugh is one hour and forty minutes, so that after one set was through, there were three in process and one being mounted in balsam. The slides were placed in each vial in the same order so that I would know that I was keeping them in consecutive order. After running through twenty slides, the xylols, the alsolute alcohols and the 95% alcohol following the Eosin were renewed. The alkaline alcohol was tested and more 2% sodium bicarbonate solution added. As the slides came from the last xylol, I laid them on clean filter paper to absorb the xylol from the lower side. Then I held them on end to let all the xylol run off that would, and absorbed with a small piece of filter paper any that was left in droplets. A drop of balsam was placed on each in the center of the sections and the cover slips placed on carefully so as to avoid air bubbles. The slides were then numbered with wax pencil and placed in an electric slide oven with the temperature regulated to 40 degrees C and left here for 36 hours to dry, then taken out and placed in the slide boxes. They were then inspected, sorted and labelled and placed in the boxes for permanent keeping.

The theory of the various solutions of the stain schedule are as follows: The xylol dissolves the paraffin and clears the sections The diminishing grades of alcohol first take the xylol out and prepare the sections for the stain which is an alcoholic stain and of about 70% strength. The tap water washes off the surplus stain and the acid and alkaline alcohols fix the stain by the formation of a salt in the tissues. The 70% alcohol again prepares the sections for the Eosin which is an alcoholic stain, the following alcohols increasing in strength dehydrate the sections preparatory to permanent mounting. The xylol

removes the alcohol and clears the sections. It is a solvent for balsam, hence combines readily with it if any is left in the sections. The balsam preserves the sections and holds the cover-glass.

DELAFIELD'S HAEMATOXYLIN STAIN

1.	Xylol (1) 10	min.
2.	Xylol (2) 10	min.
3.	Absolute alcohol (1) 5	min.
4.	Absolute alcohol (2) 5	min.
5.	95% alcohol 3	min.
6.	70% alcohol 2	min.
7.	Delafield's Haematoxylin Stain 5	min.
8.	Tap Water 2	min.
9.	Tap water 2	min.
10.	Tap water 2	min.
11.	Acid alcohol CAUTION!! Brick	red
12.	Alkaline alcohol Until	blue
13.	70% alcohol 1	min.
	Alcoholic Eosin 'Y' Stain 30	
15.	95% alcohol (1) 5	min.
16.	95% slcohol (2) 5	min.
17.	Absolute alcohol (1)	min.
18.	Absolute alcohol (2)	min.
19.	Iylol (1)10	min.
20.	Iylol (2) 10	min.
21.	Balsam	

Formula for acid alcohol

1% concentrated HCL 99% of 70% alcohol

Formula for alkaline alcohol

3 parts 70% alcohol
1 part 2% solution of acid sodium carbonate

CHARTS AND FORMS AS AIDS IN TEACHING ENTOMOLOGY

A man's efficiency depends, to a large degree, upon his training. The development of the sciences of entomology will largely be proportionate to the efficiency of the training given to the entomologists in school.

Teachers of entomology as well as teachers of other branches should study special methods to aid them in presenting the subject matter of the science.

As a student enters upon the study of insects, the field is so broad and the task so large that he will become confused unless something is done to help him to steer clear of the maze.

The subject may be approached from different angles and various devices may be used to simplify the study.

The Insect Sheet will help the student to organize the subject and call to his attention the points that are essential for him to see.

The Field Observation Sheet serves the same purpose for the student and helps the teacher to make the field trip educational instead of simply an aimless frolic.

Another helpful device is the Life Cycle Chart.

It will help to impress the mind of the student regarding the life history of insects studied.

Both the Insect Sheet and the Life Cycle Chart may

be used to good advantage on written quizzes.

Students should rear some insects and record date and observations. For this work they should be provided with an insectary record sheet on which the various points which they should observe are listed.

These few statements are merely suggestive. Many other helps and devices may be worked out and it is up to the individual teacher to be alert to discover and use anything that will improve his teaching.

Samples of the charts mentioned above are shown on the following pages.

FIELD OBSERVATION SHEET - - INSECTS

1						26	ate
Common Name of Insect	Location	Habitat	Abundance	Host	Injury	Description	General remarks
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82

INSECT SHEET

Student						I	Date	
Common Name of Insect	Order	Family	Economic Importance	Injury by Adult or larvae	Type of Metamorphosis	Mouth- parts	Control	References

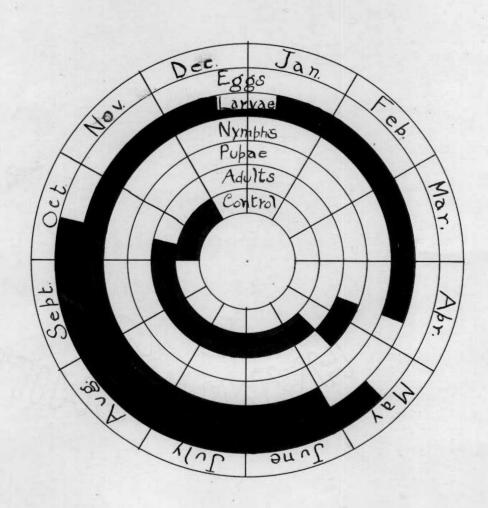
INSECTARY RECORD SHEET

Collector	Date started		
Insect	Project (Life History)		

	Date					
Cage No.	Collected	Location	Daily observations	Time	Changes	Conclutions
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INSECT LIFE CYCLE CHART



Insect	(Peach-tree	Borer)
Student_		

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