

## **SURVEY PROTOCOL FOR ASIAN CITRUS PSYLLID (ACP), *DIAPHORINA CITRI* IN CITRUS TREES**

### **• TIME OF OBSERVATION**

Citrus tree phenology during **February-March, April, May, June** and **July-August** is the appropriate phenomenal seasons to observe Asian citrus psyllid (ACP) activities including its life cycle. The population fluctuation of psyllids breeding on citrus are closely correlated with flushing rhythm, because eggs are laid exclusively on young flush points and nymphs develop on immature leaves. The heavy and prolong flushing of young trees makes them very attractive to the vector; this partly explains the rapid spread of huanglongbing (HLB) in replanted citrus groves. Moreover, peak movements of ACPs appear to occur following the spring flush of citrus foliage.

In the citrus crop cycle,

- **Mid-February to mid-March (Baishakh)** remains the critical period for spring flush and flower development.
- **Mid-March to mid-April (Chaitra)** remains the period of flowering and fruit setting.
- **Mid-April to mid-May (Baishakh)** remains developing fruits at soybean-grain-size during first week of Baishakh, and initiating summer flush development.
- **Mid-May to mid-June (Jeshth)** remains developing fruits at marble size (10 mm in diameter), and expanding summer flush during third week of Jeshth.
- **Mid-June to mid-July (Ashadh)** remains mandarin fruits increasing in size greater than a marble but lesser than a lemon size. Sweet orange fruits remain of lemon size in structure. Rainy flush may start in trees.
- **Mid-July to mid-August (Shravan)** remain expanding rainy flush in size, and mandarin fruits attaining at lemon size and sweet orange fruits at oval (egg-like) size.

### **• SELECTION OF TREES FOR OBSERVATION**

- Citrus trees with spring and rainy flushes should be selected for ACP observations typically during **April, May, June** and **July**. Relevant causes to select citrus trees in these months are illustrated in the above section of the text.

### **• NUMBER OF SAMPLE TREES UNDER OBSERVATION**

For the purpose of sampling, the field in each location should be divided into five areas each of 10 x 2 m in dimension. At weekly intervals, one shoots (about 6-10 cm long) should be selected at random from each square meter area by throwing a

pointed object. Thus a total of 20 shoots needed to select from each of the 5 areas on each sampling date. Numbers of citrus psyllid adults per shoot should be counted and recorded.

- Five places in an orchard
- Two trees in one place.
- Thus, 10 trees in an orchard.

- **PART/S OF A TREE UNDER OBSERVATION**

- Shoots each length ranging from 6 to 10 cm in twigs.
- Ten shoots in one tree. Thus, 20 shoots in one place of the orchard.
- Altogether 100 shoots needed to observe for ACPs in the orchard (5 places included in the orchard mentioned earlier)

- **FREQUANCIES OF OBSERVATIONS**

- Weekly interval

- **WAYS TO COLLECTING SAMPLE INSECT SPECIMENS**

- Observe the sample shoot and aspirate adult ACPs by means of a mouth aspirator for 5-man-minute into the glass container in built with aspirator. Count number of ACPs and print it in a data record sheet.

**Table 1. Population density of adult *D. citri* in shoot\*in orchard**

Farmer's name: xxxxxxxxxxxxxxxx xxxxxxx xxxxxxx

Locality: xxxxxxxxxxxxxxx xxxxxxx

Sampling occurrence: 1st 2 3 4 5 6 7 20th  
xxxxxxxx

Sampling date:

Sub-field→ Sample↓	Shindhuli farmer's citrus orchard number- 1						Mean ± S.E.
	1	2	3	4	5	Total	
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							
<b>Total</b>							

\*about 6-10 cm long

- **WAYS TO PRESERVING COLLECTED INSECT SPECIMENS**

ACPs sample specimens can be preserved in 1) Dry method and 2) Wet method

- **Dry preservation method:** Knock down the collected insects putting inside a polyethylene bag of 1 kg capacity contained with ethyl acetate imbibed speck of cotton. Some of the dead insects individually pinned in micro-pin at a proper height and thus prepared specimens individually mounted on a thick paper piece and the latter is pinned with collection data described in a piece of paper and placed in the display box for future process of its identification. Mentions made in collection data are 1) Host name, 2) Location name, 3) Collection date, 4) Collector name. Some of the specimens individually can be glued with quick-fix material on a piece of paper and the latter can be pinned and placed in the display box with its collection data. Pinning insect specimens should be performed while insects are fresh. Dry insects are relaxed first into a humidity container for some hours before pinning.
- **Wet preservation method:** The knock down insects are transferred into a glass vial (5-10 ml) with 70-75% alcohol and collection data described piece of paper written by means of soft pencil. Each vial should be screw capped.

- **WAYS TO PACKING INSECT SPECIMENS**

Packing insect specimens to dispatch them to a national expert and or international expert for their authentic identification or verification of their identity needs special insect care while packing. Prior consent of the national and international insect taxonomist is matter of great concern before dispatching the insects for identification. Some customary insect packing should observe the following care-takings.

Dry preserved insect specimen packing management

- Pin mounted insect specimens should be deeply pinned into the thermocole base on the bottom of wooden or stiff cardboard box. Each specimen should bear with collection data (mentioned above) and museum code (Hem., Heter., Psyl., Ent. Bio. #x) developed in the country of origin.
- Maintain ample space between pinned insects avoid collision while transporting.
- The outer surface of stout closer piece of the box must bear addresses of the sender and receiver parties.
- Packing wrapper with destination address must be durable in nature so that it should remain intact until it reaches the destination.
- Proper documents concerning to custom clearance and legal document for the quarantine clearance sent by the institute of the international insect taxonomist must be accompanied with the insect packing.
- Wet preserved insect specimen packing management
- Wet preserved insect specimens in glass vils (5-10 ml) with collection data should be put inside a wooden or stiff cardboard box. Each vial must be thickly wrapped with tissue paper to avoid damage in collision while transporting the package.

- Other requirements are as mentioned above.
- **PLACE AND PERSON/S FOR INSECT SPECIMEN DIAGNOSIS**

#### **National Institutes**

- Entomology Division, NARI, NARC, Khumaltar, Lalitpur.
- Natural History Museum, Swayambhu, Kathmandu.

#### **International Institutes**

- British Museum (Natural History), Cromwell Rd, London SW7 5BD, United Kingdom
- Commonwealth Agriculture Bureau International, Nosworthy Way, Wallingford, Oxfordshire OX10 8DE, UK