Standard Cocoa Pod Borer laboratory bioassay protocol for collaborative[1] screening of insecticides

Version: June 2006

Objective

Development of a bioassay for control of cocoa pod borer (CPB: *Conopomorpha cramerella*) that is simple and rapid, but assesses activity against both adults (indirect contact) and eggs (direct contact)

2-3 year objective: following further studies (field trials), identify effective control agents, for CPB that have a low mammalian toxicity (class III or better). Our medium term purpose is to identify a range of agents belonging to a range of mode-of-action (MOA: say 3-5 products).

Issues

Candidate insecticides, especially novel compounds with lowered environmental impact have various modes of action. For example a new insecticide might be especially effective against:

- adults
- egg fertility
- eggs
- young larvae

Procedure

1. The best time for collecting pupae is during the peak season (when up to 1000 pupae can be assembled): see further notes from UnHas.
2. Selection of control agents: a list of potentially useful products available in Indonesia and Malaysia has been prepared by Indra *et al*. This has been annotated with further information on mammalian toxicity and MOA: using the international Insecticide Resistance Action Committee (IRAC) classification.
3. Selection of dosages – based on recommended/label field rates (for other crops); concentrations:
   field dosage,
   0.32 times field dosage
   0.1 times field dosage
   control: water only
   (*e.g*. if product is recommended for use at 0.5 ml / litre of water for vegetables, test at concentrations: 0.5, 0.16 and 0.05 ml / litre of water)
4. Preparing test solutions/suspensions: standard dilution procedures to achieve the above. Particular care should be taken with the confusing labelling of insecticides (*e.g*. a product labelled "XYZ 35 EC" can contain either 35 g/L or 350 g/L). It is important to check the label carefully and use the value for g/L, g/kg or IU/kg (in the case of *Bacillus thuringiensis* products).

[1] Participants in the initial October 2005 meeting were: Adi Sumantri, Ahmad Saleh, Alias Awang, Endang Sulistyowati, Fizrul Indra Lubis, Ike Virdiana, Roy Bateman (secretary), Smilja Lambert, Stephen Nelson, Sylvia Sjam, Usniati Reni, Yonnes Hasan

5. Application:
   (a) cage 8 adults (4 females: 1-2 days old) with 1 pod
   (b) next day: mark eggs
   (c) spray pod: with 2 squeezes of a hand-sprayer: delivering not more than 1 ml formulation to the pod as a fine spray
   (d) transfer to new cage with 8 adults (4 females)
6. Assess treatments every day (at the same time) up to 7 days:
   (a) mortality of marked eggs
   (b) adult survival
   (c) new egg laying (mark daily with different shape)

Insect requirements

- Each agent/dosage – need 16 insects per rep; 5 replicates (+ 20% for handling mortality) = 100 insects.
- 400 insects needed for each agent (4 concentrations)
- Aim to screen 2 agents in one experiment

Analysis and Data presentation

A number of data analysis techniques are available for handling bioassay data: for example, insect responses can be transformed into probits, but the logistic transformation[2] can also be used legitimately (and considerably more easily with spreadsheets). The analytical approach will differ between:

a) *Fast acting (usually insect neurotoxic) molecules*: where the bioassay has a clear end point (usually in <3 days). Normal bioassay analysis techniques are applicable including the Abbott (1925) correction for control mortality, provided this does not greatly exceed 10%.

b) *Non-neurotoxic chemicals and biological agents*: which may take more than one week to show responses. A common issue here that control mortality creeps up to unacceptably high levels, and with biological agents, all doses may achieve 100% mortality given time. In these cases, average survival time statistics (*e.g*. Kaplan & Meier, 1958) are usually much more appropriate, although dose-response regressions can be interesting at certain critical periods of an assay. With Kaplan & Meier's technique (available on a number of statistical packages), mortality for a given treatment dose is expressed as an average survival time with confidence intervals to distinguish it from other treatments.

---

[2] The logit of the proportion (responding) the proportion P is transformed to value l by:

$$l = \ln \{P / (1 - P)\}$$

In practice, with percentages, division by 0 errors (0%, 100%) are avoided with:

$$l = \ln \{(P + 0.5) / (100.5 - P)\}$$