Inhibition of Esterase Mediated Hydrolysis of 1-Naphthyl Butyrate in the B-Biotype *Bemesia tabaci* (Hemiptera; Aleyroididae) in Australia

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ABSTRACT

A colourimetric squash test was used to distinguish between native and B-biotype Bemisia tabaci (Gennadius), for field identification in Australia. This test is based on overproduction of esterase iso-enzymes by B-biotype B. tabaci. This test has occasionally been shown to give a false negative due to organophosphates temporarily binding to esterase enzymes and inhibiting its reaction with an artificial substrate 1-naphthyl butyrate (Byrne and Devonshire, 1991). While it is desirable to have a quick whitefly identification test, it is also important to know if prior treatment with organophosphates will affect the results. Results obtained by polyacrylamide gel electrophoresis demonstrated that the esterase mediated hydrolysis of the artificial substrate 1-naphthyl butyrate is inhibited from 24 to 48 hours after exposure of B-biotype B. tabaci to 0.1% active ingredient organophosphate concentration on exposed leaves. This hinders the ability of the quick identification test to distinguish between resistant B-biotypes and susceptible native B. tabaci if organophosphates were used within 48 hours.

Introduction

The presence of the B-biotype *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) was first recorded in Australia in October 1994 in Darwin (Gunning *et al.*, 1995) and subsequently, became widespread (Gunning *et al.*, 1997). Preliminary evaluation of organophosphate and carbamate insecticides used against B-biotype *B. tabaci* showed high levels of resistance (Gunning *et al.*, 1997). This threatens any effective role for organophosphates and carbamates in a chemical control program (Gunning *et al.*, 1995), so sole reliance on insecticides is not conducive to long term control programmes.

Identification of *Bemisia* spp. by electrophoresis is time consuming, therefore we considered a rapid squash technique, based on total esterase. However, experience elsewhere shows that these tests occasionally give a false negative as organophosphate insecticides can temporarily bind with the esterases and inhibit reaction with an artificial substrate (Byrne and Devonshire, 1991).

The objective of this research was to determine whether organophosphate insecticides would inhibit esterases in B-biotype *B. tabaci* for any length of time, thus preventing rapid, reliable identification of the Australian B-biotype *B. tabaci*.

Materials and Methods

Insect Populations. The populations of B-biotype *B. tabaci* were maintained in a glasshouse culture on poinsettia plants (*Euphorbia pulcherrima*) at 27ºC.

Insecticides. Formulated insecticides were used for bioassays; dimethoate (10% w/v) (Rogor®), profenofos (50% w/v) (Curacron®) and fenthion (55% w/v) (Lebaycid®). They were serially diluted to the desired concentrations with distilled water.

Bioassays. Bioassays were conducted on adult *B. tabaci* on cotton (*Gossypium hirsutum*) leaves, by a technique similar to the leaf dip bioassay described by Cahill *et al.* (1995) and Byrne *et al.* (1994). *B. tabaci* were exposed to insecticide concentrations of 0% (control), 0.00001%, 0.001% and 0.1% active ingredient. *B. tabaci* were then allowed to feed on the treated cotton leaves for periods of 12, 24, 48 and 72 hours. The insects not killed by the insecticide were used in electrophoretic studies.

Polyacrylamide Gel Electrophoresis (PAGE). The methods utilized in electrophoresis were akin to those described by Byrne and Devonshire (1991). Gels were run at 250V for 2 hrs at 5ºC and stained with 0.50 mM 1-naphthyl butyrate in 0.2% Fast Blue RR salt (diazotized 4-benzoylamino-2, 5-dimethoxyaniline/ ZnCl2), prepared in 0.2M phosphate buffer pH 6.0. 1-naphthyl butyrate is an artificial substrate that is hydrolyzed by esterase enzymes and this complex was stained with Fast Blue RR salt. Gels were held in darkness at room temperature until bands were stained. After staining, they were fixed in 5% acetic acid and their images scanned, using a desktop scanner (Apple Colour One Scanner) and Apple Macintosh computer.

Results and discussion

Insects stained on the gels showed esterase patterns consistent with B-biotype *B. tabaci*, due to the presence of the distinctive esterase band at 0.14 Rm (designated as E0.14) (Gunning *et al.*, 1997). All organophosphate insecticides tested induced similar responses in esterase activity toward 1-naphthyl butyrate. Staining was absent after exposure of insects to 0.1% organophosphate concentration at 24 and 48 hours, compared to the controls. Other organophosphate concentrations did not affect *B. tabaci* esterase activity towards 1-naphthyl butyrate.

The results obtained by electrophoresis demonstrate that esterase mediated hydrolysis of the artificial substrate 1-naphthyl butyrate is inhibited for 24 to 48 hours after exposure to cotton leaves dipped in 0.1% organophosphate insecticide solutions. This corresponds to the findings that esterase activity was completely inhibited within two days in the Sudan *B. tabaci* strain confined to cotton leaves treated with 50g/ha profenofos (Byrne and Devonshire, 1991). The absence of stained esterase bands indicates that the enzymes were bound to organophosphates and were thus unable to react with 1-naphthyl butyrate.

Conclusion

This two day inhibition of esterase activity toward an artificial substrate will hinder the ability of a quick squash test to distinguish between the resistant B-biotype and susceptible native *B. tabaci*, if organophosphate insecticides have been sprayed within 48 hours. However, it is unlikely that organophosphates will be much used for the purpose of controlling *B. tabaci* B-biotype, as the insect is highly resistant to this biotype of insecticide. Despite this, organophosphorous compounds may have been applied for the purpose of controlling other pests, such as *Helicoverpa armigera* (Hübner).

This discrepancy could occasionally provide misleading information, causing managers to be unaware of the presence of B-biotype *B. tabaci* and potential problems that could develop.

Awareness of the effect organophosphate insecticides can have on the accuracy of the colourimetric squash test could lead to optimization of field whitefly diagnostic procedures and resistance management strategies.

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References

Byrne, F.J. and A.L. Devonshire. (1991): In vivo inhibition of esterase and acetylcholinesterase activities by profenofos treatments in the tobacco whitefly *Bemisia tabaci* (Genn.): implications for routine biochemical monitoring of these enzymes. Pesticide Biochem. and Physiol. 40:198-204.

Byrne, F.J., M. Cahill, I. Denholm and A.I. Devonshire. (1994): A biochemical and toxicological study of the role of insensitive acetylcholinesterase in organophosphorous resistant *Bemisia tabaci* (Homoptera: Aleyrodidae) from Israel. Bull. Ent. Res. 84:179-184.

Cahill, M., F.J. Byrne, K. Gorman, I. Denholm and A.L. Devonshire. (1995): Pyrethroid and organo-phosphate resistance in the tobacco whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae). Bull. Ent. Res. 85:181-187.

Gunning, R.V., F.J. Byrne, B.D. Condé, M.I. Connelly, K. Hergstrom, and A.L. Devonshire. (1995): First report of B-biobiotype *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in Australia. J. Australian Ent. Soc. 34:116.

Gunning, R.V., G.D. Moores and A.L Devonshire. (1996): Esterases and esfenvalerate resistance in Australian *Helicoverpa armigera* (Hübner) Lepidoptera: Noctuidae. Pesticide Biochem. and Physio. 54:12-23.

Gunning, R.V., F.J. Byrne and A.L. Devonshire. (1997): Electrophoretic analysis of Non-B and B-biobiotype *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in Australia. Australian J. Ent. 36:245-249.