Antifeedant and Antioviposition Activities of the Fractions
of Extract from a Tropical Plant, *Andrographis paniculata*
(Acanthaceae), against the Diamondback Moth,
Plutella xylostella (Lepidoptera: Yponomeutidae)¹

Wawan HERMAWAN, Shin-ichiro KAJIYAMA, Ritsuko TSUKUDA,
Kenji FUJISAKI, Akio KOBAYASHI and Fusao NAKASUJI

Faculty of Agriculture, Okayama University, Tsushima, Okayama 700, Japan

(Received 11 March, 1994; Accepted 1 August, 1994)

Antifeedant activity of extracts from *Andrographis paniculata* against the 1st and the 4th-instar larvae of the diamondback moth (DBM), *Plutella xylostella*, was tested by using leaf disc of cabbage. Furthermore, the antioviposition effect was also observed on the caged cabbage plant treated with the fractions. The active fractions from the acetone extract suppressed feeding by the 1st and 4th-instar larvae at the concentrations of 60 ppm and 500 ppm, respectively. The antioviposition activity against DBM females was also found at the concentration of 125 ppm.

Key words: antifeedant, antioviposition, *Andrographis paniculata*, *Plutella xylostella*

INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella*, is the most serious pest of cruciferous crops in many countries. The control of DBM by synthetic insecticides has become difficult because of its quickly developing resistance to these pesticides (MIYATA et al., 1986). Therefore, the use of feeding or oviposition inhibitors for control of DBM will permit the reduction of insecticide spraying and retard the development of such resistance.

In the previous study, we reported that crude extract from a tropical plant, *Andrographis paniculata*, has an effect of feeding suppress to the 4th-instar larvae of DBM (HERMAWAN et al., 1993). In the present study, we attempted to fractionate the crude extract of *A. paniculata*, and the fractions were assayed for their antifeedant activities against DBM larvae and antioviposition activities against DBM females.

MATERIALS AND METHODS

The dried powder of leaves and stems of *Andrographis paniculata* (1.34 kg) was successively extracted with *n*-hexane and acetone. The resulting *n*-hexane extracts were concentrated (10 g), and subjected to an open column chromatography (4.0 cm

¹ This work was supported in part by the Scientific Cooperation Program under the Core University (Nagoya University) System of the Japan Society for the Promotion of Science (JSPS) and Grant-in-Aid for Scientific Research No. 06454062 from the Ministry of Education, Science and Culture (Mombusho), Japan.

×48 cm; packing; Wakogel C-100), and eluted stepwise with 1,000 ml of 0, 5, 10, 15, 20, 30, 40, 50, 70 and 100% of ethyl acetate in *n*-hexane to give 10 fractions (Hfr. 1: 12.3, Hfr. 2: 660, Hfr. 3: 302, Hfr. 4: 438, Hfr. 5: 57.2, Hfr. 6: 82.2, Hfr. 7: 84.9, Hfr. 8: 20.3, Hfr. 9: 16.9 and Hfr. 10: 8.0 mg). On the other hand, the acetone extracts (30.5 g) was also subjected to an open column chromatography (6.5 cm×40 cm; packing; Wakogel C-100), and eluted stepwise with 1,000 ml of 0, 10, 20, 30, 40, 50, 70 and 100% of acetone in benzene and 100% of methanol to give 9 fractions (Afr. 1: 62.2, Afr. 2: 443, Afr. 3: 233, Afr. 4: 934, Afr. 5: 726, Afr. 6: 693, Afr. 7: 27, Afr. 8: 443, and Afr. 9: 1,047 mg). Each fraction was evaporated *in vacuo*, weighed, and diluted to various concentrations from 60 ppm to 1,000 ppm with methanol in order to prepare the sample solution for antifeedant and antioviposition tests. Except the 100% benzene fraction, rest of the fractions were subjected to the biological test, because the 100% benzene fraction was oily and the leaf discs were severely damaged. The fractionation procedure was shown in Fig. 1.

Antifeedant test for DBM larvae. A leaf disc was prepared from commercial cabbage by the cork borer. The leaf disc (3 cm in diameter) was dipped into test solution for 10 sec and the excess solution was dried before being placed on wet filter paper in an ice cream cup (6.5 cm in diameter). Another disc was treated similarly with 50% methanol in water (control). The 4th-instar larvae of DBM were obtained from a stock culture. Two larvae were fed on the leaf disc for 24 h at 25°C under 16L-8D. The area of leaf disc before and after feeding was measured using a digitizing leaf area meter (Hayashi Denkoh, Model AAM-8). The leaf area consumed was modified with respect to the initial area of 7.065 cm². Ten replications were made for each treatment and the control group. The same experimental design was used to test the antifeedant activity for the 1st-instar larvae of DBM. Since the amount of feeding by the 1st-instar larvae was small, the number of feeding traces was counted. Ten 1st-instar larvae were fed on the disc for 24 h.

Fig. 1. Schematic representation of the procedure of extraction and fractionations.

Antioviposition test for DBM females. A single potted cabbage plant (stage 5–6 leaves; variety: Kogetsu of Takii Shubyo) was placed in a cylindrical cage (12 cm in diameter and 12 cm in height) with screen on the top. The whole plant had been sprayed with each sample. Three one-day-old DBM females which had been mated were released into this cage. The number of eggs laid within 24 h was recorded. The control group was treated with 50% methanol in water. Five replications were made for each treatment.

RESULTS

Figure 2 shows the mean value of consumed area (cm^2) of the leaf disc by two individuals of the 4th-instar larvae in 24 h at 1,000 ppm of all fractions from *n*-hexane and acetone extract. The area consumed was not significantly different at all fractions in *n*-hexane extract. Two fractions (Afr. 5 and Afr. 7) significantly suppressed feeding of the DBM larvae ($p < 0.05$, DUNCAN's multiple range test). In the fraction 5 and 7, the leaf consumption decreased to one third (Afr. 5) and one half (Afr. 7) as compared with that of control.

Figure 3 shows the mean value of consumed area of the leaf disc by the 4th instar larvae within 24 h at different concentrations of the Afr. 5 and Afr. 7. The 500 ppm of the both fractions had a similar activity of feeding suppression and the leaf area consumed at 500 ppm was about one half of the control. The effect on feeding suppression became weak at 250 ppm in both the fractions.

Figure 4 shows the number of feeding traces by 10 individuals of the 1st-instar

Fig. 2. Comparison of leaf area consumed by two individuals of the 4th-instar larvae of DBM for 24 h at each fractions from *n*-hexane and acetone extracts. All fractions are 1,000 ppm. Bars with the same letters are not significantly different at $p = 0.05$ between the fraction and the control (DUNCAN's multiple range test).

Fig. 3. Comparison of leaf area consumed by two individuals of the 4th-instar larvae of DBM for 24 h at different concentrations of Afr. 5 and Afr. 7 from acetone extract (see Fig. 2). Bars with the same letters are not significantly different at $p=0.05$ between the fraction and the control (DUNCAN's multiple range test).

Fig. 4. Comparison of feeding traces by 10 individuals of the 1st-instar larvae of DBM for 24 h at different concentrations of Afr. 5 and Afr. 7 from acetone extract (see Fig. 2). Bars with the same letters are not significantly different at $p=0.05$ between the fraction and the control (DUNCAN's multiple range test).

larvae of DBM for 24 h. The number was significantly small at every concentration of both the fractions as compared with that of the control ($p<0.05$). All larvae survived after 24 h in each of the experiments mentioned above.

Figure 5 shows the number of eggs laid by DBM females on the cabbage plant treated with the fractions of the acetone extract for 24 h. The number of eggs laid was

Fig. 5. Comparison of the number of eggs laid by three females of DBM for 24 h at different concentrations of Afr. 5 and Afr. 7 from acetone extract (see Fig. 2). Bars with the same letters are not significantly different at $p=0.05$ between the fraction and the control (DUNCAN's multiple rang test).

significantly small at the concentrations of 125 and 250 ppm in the Afr. 5 and Afr. 7 as compared with that in the control ($p<0.05$). The both fractions seemed to give a similar effect on egg laying. The effect of antioviposition became weak at 60 ppm.

DISCUSSION

Our previous study (HERMAWAN et al., 1993) reported that the crude methanol extract from *A. paniculata* suppresses the feeding of the 4th-instar larvae of DBM. In the present study, we found that the extract also suppressed the oviposition by the female adults of DBM. When the crude extract was treated, the minimum concentration for antifeedant activity against the 4th-instar larvae was 2,500 ppm (HERMAWAN et al., 1993). On the other hand, the active fractions originated from acetone extract (Afr. 5 and Afr. 7) suppressed the feeding by the 4th-instar larvae at 500 ppm, and also suppressed the feeding by the 1st-instar larvae as low as 60 ppm. The antioviposition activity against DBM females was found at the concentration more than 125 ppm. Therefore, if the active compound in the fractions could be purified, the activity level might increase more than that mentioned above.

The insect feeding deterrents have been investigated on various plants (JACOBSON et al., 1978). Especially, the extracts from Neem, *Azadirachta indica*, have antifeedant activities against many kinds of insects (LOWERY and ISMAN, 1993) although a clear evidence of antifeedant effects against DBM has not been obtained. Antifeedant effects of extracts from *Lycopersicum esculentum* and from *Ajuga chamaepibys* on DBM larvae were reported by THORSTEINSON (1953) and GRIFFITHS et al. (1991), respectively. DAIDO et al. (1993) found that several quassinoids which were obtained from *Picrasma*

ailanthoides have antifeedant activity against DBM. The α -tomatine also reduced feeding of DBM larvae (LU and CHU, 1992). As mentioned above, there are a few studies on the antifeedant activities of substances from plants on DBM. In the future studies, we will attempt to purify the active substances from *A. paniculata*.

ACKNOWLEDGEMENTS

The authors thank to Messrs. Y. KOSHIYAMA, S. ONISHI, K. SASAKI, K. HAGIHARA, and K. UKITA, Faculty of Agriculture, Okayama University for assistance in our experiment. Thanks are also due to Prof. S. SUDARWATI, Bandung Institute of Technology, Indonesia, Prof. Dr. H. ISHIZAKI and Dr. M. KAWAKAMI, Nagoya University, for their help in the JSPS program.

REFERENCES

- DAIDO, M., N. FUKAMIYA, M. OKANO, K. TAGAHARA, M. HATAKOSHI and H. YAMAZAKI (1993) Anti-feedant and insecticidal activity of quassinoids against diamondback moth (*Plutella xylostella*). *Biosci. Biotech. Biochem.* **2**: 244–246.
- GRIFFITHS, D.C., S.P. MANIAR, L.A. MERRITT, A. MUDD, J.A. PICKETT, B.J. PYE, L.E. SMART and L. WADHAMS (1991) Laboratory evaluation of pest management strategies combining antifeedants with insect growth regulator insecticides. *Crop Protection* **10**: 146–151.
- HERMAWAN, W., R. TSUKUDA, K. FUJISAKI, A. KOBAYASHI and F. NAKASUJI (1993) Influence of crude extracts from a tropical plant, *Andrographis paniculata* (Acanthaceae), on suppression of feeding by the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae) and oviposition by the azuki bean weevil, *Callosobruchus chinensis* (Coleoptera: Bruchidae). *Appl. Entomol. Zool.* **28**: 251–254.
- JACOBSON, M., D.K. REED, M.M. CRYSTAL, D.S. MORENO and E.L. SODERSTROM (1978) Chemistry and biological activity of insect feeding deterrents from certain weed and crop plants. *Entomol. Exp. Appl.* **24**: 248–257.
- LOWERY, D.T. and M.B. ISMAN (1993) Antifeedant activity of extracts from Neem, *Azadirachta indica*, to strawberry aphid, *Chaetosiphon fragaefolii*. *J. Chem. Ecol.* **8**: 1761–1773.
- LU, F.M. and Y.I. CHU (1992) Antifeeding effects of α -Tomatine on larvae of the diamondback moth (*Plutella xylostella* L.). *Chinese J. Entomol.* **12**: 135–145.
- MIYATA, T., T. SAITO and V. NOPPUN (1986) Studies on the mechanism of diamondback moth resistance to insecticides. In *Diamondback Moth Management* (N.S. TALEKAR, ed.). AVDRC Publication, Taiwan, pp. 347–357.
- THORSTEINSON, A.J. (1953) The chemotactic responses that determine host specificity in oligophagous insect (*Plutella maculipennis* (CURT.): Lepidoptera). *Can. J. Zool.* **31**: 52–72.