Insecticide Resistance of the Cotton
Aphid, *Aphis gossypii* GLOVER
(Homoptera: Aphididae)
VI. Qualitative Variations of Aliesterase
Activity

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Resistance to organophosphorus insecticides is positively correlated with the high aliesterase (AliE) activity in the cotton aphid, *Aphis gossypii* (SUN et al., 1987; HAMA and HOSODA, 1988; TAKADA and MURAKAMI, 1988; SAITO, 1989, 1990b). Such quantitative variation of AliE activity is also associated with host plant preferences of the aphid, i.e. aphids with high AliE activity are frequently observed on Cucurbitaceae crops but rarely on Solanaceae (HAMA and HOSODA, 1988; TAKADA and MURAKAMI, 1988; SAITO, 1990 a, 1991). The present paper evaluates qualitative variations of AliE activity in aphids from various plants by isoelectric focusing (IEF).

MATERIALS AND METHODS

Sixteen clonal strains and two nonclonal strains of *A. gossypii* collected from 12 species of plants in Shizuoka Prefecture were tested (Table 1). All clonal strains were maintained on excised strawberry leaves in glass tubes (25 mm dia. $\times$ 12 cm ht.) at 20°C. The nonclonal strains collected from a shoot of *Celastrus orbiculatus* and *Rubia akane* were used directly because they could not be reared on strawberry leaves in the laboratory. The total AliE activity and the total protein of aphids tested were determined using whole body homogenates of adult apterous viviparae by Asperen's method (HAMA and HOSODA, 1988) and Bradford's method (Tojo, 1980) with bovine albumin as a standard protein, respectively.

For analysis of AliE isozyme, adult apterous

viviparae of each strain were homogenized in 1/15 M phosphate buffer (pH 7.2), then 20 μ l of each homogenate was applied onto a piece of filter paper on a precast ampholine polyacrylamide gel plate (pH 3.5–9.5; Pharmacia LKB, Sweden). The number of individuals per sample (1 to 50 aphids/100 μ l of buffer) was adjusted depending on the degree of total AliE activity. The IEF (Model Resolmax-AE 3230; Atto, Japan) was run for 30 min at a constant power of 5W, and then for 3 h at 10W after removing the filter paper. The gel was kept cool during the operation of the machine by circulating cold water. The AliE isozyme pattern was detected by staining with 0.1% Fast Blue RR in 1/10 M phosphate buffer (pH 6.2) containing 0.03% α -naphthyl acetate. The stained bands on the gel were scanned at 600 nm using a densitometer (Model CS-910; Shimadzu, Japan).

The isoelectric point (pI) of each band was estimated by the pI marker proteins (pI range 4.7–10.6; LKB, Sweden), which were run in parallel with the samples on the gel.

RESULTS AND DISCUSSION

On the basis of the AliE zymograms detected by IEF, the *A. gossypii* strains tested could be classified into three types, E-1, E-2, and E-3 (Fig. 1, Table 1). Type E-1 had three active bands of pI 5.5, 5.8, and 6.0, and two minor ones of pI 5.3 and 5.4. Type E-2 had three active bands of pI 5.3, 5.4, and 6.0, and three minor ones of pI 5.25, 5.5, and 5.75. The broadening band of pI 6.0 detected in types E-1 and E-2 seemed to consist of at least two active bands, and its activity differed by strains and among experimental replications in the same strain. It was possible to distinguish type E-1 from type E-2 by the existence of a band of pI 5.8. In type E-3 there were three bands of pI 5.27, 5.35, and 7.5, none of which was detected in type E-1 or E-2.

As shown in Table 1, strains with comparatively high AliE activity belonged to type E-1, except for the aphid collected from *C. orbiculatus*. By contrast, strains with low AliE activity belonged to type E-2, regardless of their host plant. The AliE isozyme pattern did not change when the aphids were transferred from their original host plants to a cultural strawberry host plant (data not shown). Nor was the AliE activity of the aphid altered by changing host plants (SAITO, 1991).

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Table 1. Origins, AliE activity, and AliE isozyme patterns of *A. gossypii* strains collected in Shizuoka Prefecture

Strain	Date collected	Original host plant	AliE activity (nmol/10 min/ μ g protein)	Type of AliE isozyme pattern ^a
C1	Aug. '88	<i>Cucumis sativus</i>	103	E-1
M2	Oct. '88	<i>Cucumis melo</i>	79	E-1
Ch4	Aug. '89	<i>Chrysanthemum morifolium</i>	75	E-1
St1	Aug. '88	<i>Fragaria grandiflora</i>	57	E-1
Man2	Apr. '91	<i>Citrus unshiu</i>	55	E-1
VM6	Apr. '88	<i>Veronica persica</i>	51	E-1
Pe2	Apr. '91	<i>Pyrus serotina</i> var. <i>culta</i>	50	E-1
P2	Jun. '88	<i>Solanum tuberosum</i>	46	E-1
H10	May '89	<i>Hibiscus syriacus</i>	34	E-1
E10	Aug. '89	<i>Solanum melongena</i>	30	E-1
Cel ^b	Apr. '91	<i>Celastrus orbiculatus</i>	4	E-1
E1	Sep. '88	<i>Solanum melongena</i>	7	E-2
P1	May '88	<i>Solanum tuberosum</i>	7	E-2
Ch5	May '88	<i>Chrysanthemum morifolium</i>	7	E-2
HE2	May '88	<i>Hibiscus syriacus</i>	6	E-2
VE1	May '89	<i>Veronica persica</i>	3	E-2
St32	Apr. '91	<i>Fragaria grandiflora</i>	3	E-2
Rub ^b	Jun. '91	<i>Rubia akane</i>	2	E-3

^a See Fig. 1.^b indicates nonclonal strain. Fig. 1. Three types of AliE isozyme patterns in *A. gossypii* by isoelectric focusing. Values indicate the isoelectric point (pI) of each band.

Only the aphid obtained from *R. akane* was type E-3. The biotype of *A. gossypii* living on *R. akane* has a non-migratory life cycle in Japan (INAZUMI, 1981). Therefore, Japanese *A. gossypii* species infesting agricultural crops are probably either type E-1 or E-2.

The author had previously measured the individual AliE activity of ca. 3,700 Japanese *A. gossypii* collected from various kinds of plants, and divided the aphids into groups based on their AliE activities: higher or lower than a discriminating value, 30 to 40 nmol/10 min/ μ g protein (SARTO, 1990a). It

may thus be possible to discriminate between E-1 and E-2 by this AliE activity. The qualitative variation of AliE activity is apparently associated with its quantitative variations. Recently O'BRIEN et al. (1992) found that IEF of AliE showed both qualitative and quantitative differences in banding patterns of susceptible and resistant *A. gossypii*. Their results are similar to the present ones.

On the other hand, TAKADA and MURAKAMI (1988) classified the AliE isozyme pattern of Japanese *A. gossypii* into six types, using polyacrylamide gel electrophoresis. They stated that all tested clones with high AliE activity showed the same zymogram, called T-1. Type T-1 probably correspondings to type E-1 in the present study, and all, or some, of their other five types with low AliE activity may be part of the type E-2. FURK et al. (1980) found that pirimicarb resistant strains of *A. gossypii* showed a zymogram of AliE different from susceptible ones by IEF in England. However, no banding pattern observed in the present experiment was similar to any of the English strains.

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