Observation on the effectiveness of a novel repellent, acetylated glyceride, against the adult and the progeny of sweet potato whiteflies, *Bemisia tabaci*

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The effectiveness of acetylated glyceride (acetic and fatty acid esters of glycerol) developed as an adult repellent was clarified by observing the host selection behavior of the sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), on treated plant leaves. In a choice test between treated and untreated grape tomato leaves, it was observed that almost the same number of adults landed on the leaves, but the retention rates of adults settling under the leaves were 27% and 95%, respectively. Repellent behavior was induced within 5 sec of the adult's landing on the plant surface. The repellent led to a large reduction in the number of eggs laid on grape tomato leaves as compared to untreated leaves. However, no adulticidal activity by direct foliar treatment and no insecticidal activity against the progeny by contact treatment were found on cucumber leaves. © Pesticide Science Society of Japan

**Keywords:** acetylated glyceride, *Bemisia tabaci*, host selection, food additive, repellent.

**Introduction**

The sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is known to have an extremely broad range of host plants (over 600 species) in tropical and subtropical areas.1–3) Phytotoxic damage is directly caused by large numbers of adult whiteflies feeding on the phloem sap of squash,4) pumpkins,5) Brassica spp.,6) and tomatoes,7) as well as indirectly by the production of large amounts of honeydew excreta, which induces the growth of saprophytic fungi on foliage and fruit.

*B. tabaci* can complete 16–17 generations each year in favorable climate conditions.8) In addition, the number of eggs laid by a female varies from 48 to 394 eggs on cotton in Egypt, depending on the temperature and the host species.9) This high reproductive potential partly explains the rapid development of pesticide resistance in whiteflies.9) To avoid plant damage caused by whiteflies and the further development of chemical resistance, it is important to suppress increases in the progeny population, particularly in crops that are cultivated for long periods, such as tomatoes and eggplants.

We discovered that acetylated glyceride (acetic and fatty acid esters of glycerol), which is a food additive, have a highly repellent effect on adults of *B. tabaci* (B- and Q-biotypes), but without having phytotoxic effects on the target crops or causing toxicity in natural enemies and pollinators. After successive foliar treatments with 0.2% (v/v) acetylated glyceride 2 or 3 times at 7-day intervals, we observed a large reduction in whitefly adults, courting pairs, and nymph populations on eggplant leaves in a plastic greenhouse.10)

In the present study, we observed the effects of treatment with acetylated glyceride on the process of host selection behaviors and morphological characteristics of their labia and oviposition behaviors of *B. tabaci* adults. In addition, we evaluated the impact of the treatment on various developmental stages of *B. tabaci* in laboratory conditions.

**Materials and Methods**

1. **Acetylated glyceride**

The acetylated glyceride was formulated as a solution with an 80% emulsifiable concentrate (EC). In the present study, treatment with acetylated glyceride was conducted without the addition of a spreader.

2. **Insects tested**

*B. tabaci* (B-biotype) were reared on potted cabbage plants in growth chambers at 25°C ± 1°C with approximately 70% relative humidity and a light:dark photoperiod cycle of 16:8 hr.
3. Observation of adult behaviors
Adult whiteflies select a possible host plant on the basis of visual cues rather than odors, and they generally land on the upper side of leaves. Based on these characteristic behaviors, the host selection of adult whiteflies was observed after the upper side of host leaves were treated with acetylated glyceride.

4. Host selection behaviors and oviposition
4.1. Observation of host selection processes
Grape tomato seedlings (cv. Yellow pear) were raised until the three-true-leaf stage in plastic pots (6.5 cm in diameter, 7.5 cm in height). To obtain seedlings with only the third true leaf, other leaves, new shoots, and seed leaves were removed, and the plants were left for one day. Solutions of acetylated glyceride (0.2%, v/v) or water were sprayed onto the seedlings, using a hand sprayer with run-off. After drying for a few hours in the dark, the seedlings were placed side by side in a clear plastic cage (26 cm in length, 34 cm in width, 34 cm in height). The cage was made of vinyl chloride, and its three sides were covered with a fine woven polyethylene mesh to allow ventilation. Furthermore, a small piece of cucumber leaf bearing several B. tabaci adults (B-biotype; sex-mixed; 3–6 days after emergence) was placed upside down as an inoculation source at the same distance and height from both test pots in a cage. The adults naturally flew to determine a suitable host over time. Five replicates were performed.

In these choice tests, all adult behaviors were recorded for 1 hr using a video camera (Panasonic, model NV-DJ100) after the first individual landed on the upper sides of leaves. After carefully watching the recorded video tapes, the numbers of adults on the leaves were classified according to the following four groups: 1, an adult landed on the upper side of leaves; 2, an adult flew away after landing on the upper side of leaves; 3, an adult moved to the underside of leaves and settled after landing; 4, an adult remained on the underside of leaves at the end of the observation. The latter value included the numbers of adults that directly flew to and settled on the underside of leaves. In addition, we measured the required response time for all individuals between groups 1 and 2 (from landing to flying away) and between groups 1 and 3 (from landing to the initiation of movement).

4.2. Impact on oviposition of the repellent effect
Grape tomato seedlings (cv. Yellow pear) at the two-true-leaf stage were cut back to remove a new shoot and the two seed leaves to retain both the first and second true leaves before transferring into small plastic cups (2 cm in diameter, 4 cm in height) filled with water. The tops of the cups were sealed with parafilm. A solution of acetylated glyceride (0.2%, v/v) was carefully sprayed onto either the first or second leaf using a handy sprayer; the remaining leaf that was not sprayed was retained as an untreated control. After drying, the seedlings in the cup were placed into glass test tubes (4 cm in diameter, 13 cm in height). B. tabaci (B-biotype) females aged 3–5 days after emergence were released into the test tubes for 7 days. The numbers of settled adults on the treated leaf, untreated leaf, and places other than the leaf (e.g., the stem and the side of the tube) were counted each day. At 7 days after release (DAR), the numbers of eggs laid were counted on both leaves. In total, 21 glass tubes were tested with one replicate.

5. Impact on various developmental stages of B. tabaci
5.1. Settled adults by direct foliar treatment
The adulticidal activity of B. tabaci (B-biotype; sex-mixed) adults following direct foliar treatment with acetylated glyceride was assessed in this test. Cucumbers (cv. Hokushin) were grown in the plastic pot until the first true leaf stage. They were placed in a rearing cage for 24 hr at 25 ± 1°C to ensure adult settling on leaves. After settled adults were counted, plants were individually placed in a whitefly-free cage. Approximately 0.2 mL of acetylated glyceride solution (0.2%, v/v) or water was applied to each leaf once using a hand sprayer. Immediately after treatment, untreated control plants were placed as adult shelters in all treatments. The numbers of dead adults on the both treated and untreated plants were counted 24 hr after treatment. The experiments were performed with 6–7 replicates.

5.2. First-instar nymphs by contact treatment
Cucumbers (cv. Hokushin) were grown in plastic pots until the first-true-leaf stage. A small plastic container covered with a fine mesh for ventilation (15 mm in diameter and height) was attached to the underside of the leaf. Approximately 20 B. tabaci (B-biotype) females per plant were released into the case and allowed to lay eggs freely for 15 hr. Five days after oviposition, acetylated glyceride solution (0.2%, v/v) or water was applied to all leaves except those beneath the container (oviposition site) using a brush. After hatching, the first-instar nymph, called the clawer stage, generally walks around the leaf to find a suitable settling site. From 31 to 38 target individuals were selected for evaluation and were settled in treated areas outside the oviposition sites. The experiments were performed with 3 replicates (treated) and 4 replicates (untreated). Stereoscopic microscopic observations were made at three developmental stages of the whitely: early-instar nymphs (primarily second-instar nymphs 14 days after treatment [DAT]), late-instar nymphs (primarily fourth-instar nymphs 22 DAT), and exuviae 26 DAT.

6. Data analysis
The numbers of adults that landed and settled on leaves (section 4.1.), of eggs laid (section 4.2.), of living and dead adults on the treated leaves following direct foliar treatment (section 5.1.), and of dead first-instar nymphs by contact treatment (section 5.2.) were analyzed using t-tests at α = 0.05.

Results
1. Adult repellent behaviors and impact on oviposition
1.1. Observation of repellent processes
The mean (± standard error, S.E.) numbers of B. tabaci (B-biotype) adults that landed on the upper sides of acetylated glyceride-treated and untreated leaves were 20.0 ± 4.6 and 18.8 ± 2.5, respectively (p > 0.05, t-test; Fig. 1). In contrast, 1 hr after releas-
In these choice tests, the total numbers of adults that landed on the undersides of treated leaves (5.2 ± 1.7) were approximately 82% lower than those on the untreated controls (28.2 ± 3.2) (p < 0.05, t-test; Fig. 1).

In these choice tests, the total numbers of adults that landed on the undersides of treated leaves (5.2 ± 1.7) were approximately 82% lower than those on the untreated controls (28.2 ± 3.2) (p < 0.05, t-test; Fig. 1).

Fig. 1. Effects of treatment with acetylated glyceride on the landing and settling of *Bemisia tabaci* (B-biotype) adults on grape tomato leaves. Each value is the mean ± standard error (S.E.) based on five replicates. Asterisks (*) denote values that significantly differed from those of untreated leaves (p < 0.05, t-test).

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Fig. 2. Effects of treatment with acetylated glyceride on the behaviors of *Bemisia tabaci* (B-biotype) adults and on the response time required for conducting subsequent actions after landing on grape tomato leaves. Each value is the total number of adults observed from five replicates.

Fig. 3. The effect of treatment with acetylated glyceride on the settling of *Bemisia tabaci* (B-biotype) adults on grape tomato leaves in a choice test. Twenty-one glass tubes were tested with one replicate.

Fig. 4. The effect of treatment with acetylated glyceride on oviposition by *Bemisia tabaci* (B-biotype) adults on grape tomato leaves in a choice test. Each value is the mean ± S.E. based on 21 glass tubes with one replicate. The result is significantly different from those obtained with untreated leaves (p < 0.05, t-test).

Fig. 5. The effect of treatment with acetylated glyceride on the settling of *Bemisia tabaci* (B-biotype) adults on grape tomato leaves in a choice test. Twenty-one glass tubes were tested with one replicate.
Table 1. Effect of direct acetylated glyceride treatment (0.2%, v/v) on settled adults of *Bemisia tabaci* (B-biotype) on cucumber leaves

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Percent mortality (mean ± S.E.) at 24 hr</th>
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</thead>
<tbody>
<tr>
<td>Acetylated glyceride</td>
<td>2.1 ± 0.7</td>
</tr>
<tr>
<td>Water (untreated)</td>
<td>3.6 ± 0.8</td>
</tr>
</tbody>
</table>

Values are presented as means ± S.E. of 6–7 replicates. The number of adults settled on the treated plot and untreated plot were 107 ± 14 and 88 ± 5, respectively at pretreatment. No significant differences were observed between treatments (arcsine transformation before analysis; *p* > 0.05; *t*-test).

Table 2. Effect of acetylated glyceride contact treatment (0.2%, v/v) on first-instar nymphs of *Bemisia tabaci* (B-biotype) on cucumber leaves

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Percent mortality (mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14 DAT</td>
</tr>
<tr>
<td>Acetylated glyceride</td>
<td>5.9 ± 5.2</td>
</tr>
<tr>
<td>Water (untreated)</td>
<td>8.0 ± 9.3</td>
</tr>
<tr>
<td></td>
<td>22 DAT</td>
</tr>
<tr>
<td>Acetylated glyceride</td>
<td>11.0 ± 11.6</td>
</tr>
<tr>
<td>Water (untreated)</td>
<td>11.9 ± 14.9</td>
</tr>
<tr>
<td></td>
<td>26 DAT</td>
</tr>
<tr>
<td>Acetylated glyceride</td>
<td>14.4 ± 12.5</td>
</tr>
<tr>
<td>Water (untreated)</td>
<td>11.9 ± 14.9</td>
</tr>
</tbody>
</table>

Values are presented as means ± S.E. of 3–4 replicates. No significant differences were observed between treatments (arcsine transformation before analysis; *p* > 0.05; *t*-test).

(± S.E.) numbers of eggs laid on treated leaves (4.1 ± 1.4) were significantly reduced by 85.6% as compared with those on untreated leaves (28.4 ± 2.5) (*p* < 0.05, *t*-test). No dead females were observed during the 7 days. The significant reduction of egg numbers was caused by lower numbers of female adults settling on the treated leaves.

2. Impact on various developmental stages by direct/contact treatment

2.1 Settled adults by direct foliar treatment

No adulticidal activity was observed with direct foliar treatment of acetylated glyceride 24 hr after treatment (*p* > 0.05, *t*-test; Table 1).

2.2 First-instar nymphs by contact treatment

Mean mortalities with acetylated glyceride treatment and the untreated control were 14.4% and 11.9%, respectively, 26 DAT. There was no significant difference between treatments (*p* > 0.05, *t*-test; Table 2).

Discussion

Observations of adult behaviors in the choice test showed that the numbers of adults that landed on treated and untreated leaves were almost the same. Of the adults that landed on a leaf, 64% (46/72) adults immediately flew away from the treated leaves, whereas no (0/5) adults flew away from untreated leaves. As a preliminary observation, we observed that adults extended their labia to touch the leaf surface within 5 sec after landing on both treated and untreated leaves. In agreement with our observations, the adult bayberry whitefly *Parabemisia myricae* and the greenhouse whitefly *Trialeurodes vaporariorum* are known to touch, rub, and tap the apex of their labia on a plant surface immediately before inserting their stylets into the plant tissues.

Electrophysiological measurements obtained using an electronic monitoring system showed that a specific waveform lasting 3–15 sec was recorded before they inserted their stylets, which occurred during the establishment of electrical contact between the *B. tabaci* (B-biotype) adult’s mouthparts and the plant tissues. We guess that the adult behaviors exhibited immediately after landing may be related to the acquisition of plant surface information regarding potential hosts. Several terpenoids, including sesquiterpenes and monoterpenes, are observed in tomato accessions and ginger oil, which have putative repellent effects on *B. tabaci* adults in the vapor phase. However, the repellent effect of treatment with acetylated glyceride seems not to be caused by the vapor phase but to be primarily attributable to interference with cues associated with host recognition immediately after adults land on plant surfaces.

After adult whiteflies settle on a host plant that is suitable for both feeding and mating, they rarely fly away from the host plant unless host deterioration occurs or an external stimulus, such as bumping, is received. Therefore, the large reduction in the egg numbers in the choice test was mainly caused by the reduced numbers of settling adults due to the repellent effect.

There was no adulticidal activity by direct foliar treatment and no insecticidal activity on first-instar nymphs by contact treatment in this study. Only early-instar nymph populations was significantly reduced, by approximately 50% of those of the untreated control, when direct foliar treatment with acetylated glyceride was made on the different developmental stages—eggs, early-, and late-instar nymphs of *B. tabaci* (B-biotype)—in our previous study. Treatment with acetylated glyceride seems to act as a spiracle-blocking substance on early-instar nymphs, partly because its active ingredient is classified as a fatty acid ester of glycerol, as is one of the other spiracle-blocking insecticides.

In conclusion, the repellent effect of treatment with acetylated glyceride on *B. tabaci* adults was induced immediately after they landed on the plant surface, resulting in a large reduction in the number of eggs on leaves. On the basis of our present and previous results, no insecticidal activity against all developmental stages of *B. tabaci*, except for early-instar nymphs, was revealed with treatment with acetylated glyceride.

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References