TECHNIQUES FOR DETECTING *ACTINIDIA* RESISTANCE TO LEAFROLLERS

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ABSTRACT

Hayward and Hort16A kiwifruit are susceptible to attack by the brownheaded leafroller (BHLR), *Ctenopseustis obliquana*, but the incidence and severity of damage to Hayward can be twice that of Hort16A. Three bioassay techniques were tested for their ability to detect differences in the relative susceptibility of the two kiwifruit cultivars to BHLR larvae. No differences were detected when larvae were reared on artificial diets containing Hayward or Hort16A plant material. Significantly more larvae survived when reared on Hayward versus Hort16A leaf discs. Caging larvae onto leaves and fruit resulted in significantly more damage to Hayward compared with Hort16A. Measuring larval survival after 21 days on leaf discs, or the incidence and severity of damage caused by larvae caged on leaves or fruit, are both potential techniques for screening *Actinidia* plant material for resistance to leafrollers.
Keywords: leafrollers, kiwifruit, host plant resistance, selection tools.

INTRODUCTION

Leafroller caterpillars (Lepidoptera: Tortricidae) are the most damaging pests of kiwifruit in New Zealand. At least six species of leafroller can be found on kiwifruit vines including the brownheaded leafrollers (BHLR), *Ctenopseustis obliquana* (Walker) and *C. herana* (Felder and Rogenhofer); the greenheaded leafrollers (GHLR), *Planotortrix excessana* (Walker) and *P. octo* Dugdale; the black-lyre leafroller (BLLR), *Cnephasia jactatana* (Walker); and the lightbrown apple moth (LBAM), *Epiphyas postvittana* (Walker). All species are endemic with the exception of LBAM. The damage caused by the different leafroller species is indistinguishable, but their relative importance as pests varies according to their abundance, whether they feed primarily on leaves or fruit, the age of the plant material, and the host *Actinidia* species or cultivar.

BHLR is the dominant pest species on both *Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson cv. Hayward and *Actinidia chinensis* Planch cv. Hort16A (McKenna 1997), with most damage occurring in the 8 weeks immediately after fruit set (Stevens et al. 1995). However, the incidence and severity of damage to Hayward can be twice that of Hort16A (McKenna 1997; McKenna et al. 1998*)*. Other *Actinidia* species also vary in their susceptibility to leafrollers (Hill 2004), indicating that resistance mechanisms are present in *Actinidia*.

In this study a simple bioassay was sought that could reliably be used to screen *Actinidia* leaves and fruit for resistance to leafrollers, and which could reduce or eliminate the need for labour-intensive field monitoring. Three techniques were compared for their ability to detect differences in the relative susceptibility of Hayward and Hort16A to attack by BHLR larvae. Bioassays 1 and 2 measured antibiosis, whilst in bioassay 3 plant damage was measured. Resistance resulting from antixenosis was not evaluated although it could also be a factor in the field.

METHODS

Plant material and insects

Kiwifruit vines were located in a block at the Te Puke HortResearch Centre, trained on a T-bar structure, and received standard vine husbandry except that no bud-burst enhancers or pesticides were applied throughout the season. The Hort16A vines were 7 years old and the Hayward vines 20 years old in 2004. Five Hort16A and five Hayward vines were selected without conscious bias in September 2004. BHLR larvae for the bioassay tests came from the 9th and 10th generation of a laboratory colony originating from Te Puke.

Bioassay 1 – artificial diets

Forty leaves and 50 fruit were collected from each of the tagged vines at approximately 6 weeks after bud-burst and 4 weeks after fruit set respectively. Actual collection dates were: Hort16A leaves, 10 November 2004; Hayward leaves, 22 November 2004; Hort16A fruit, 29 November 2004; and Hayward fruit, 5 January 2005.

Plant material from each vine was combined and freeze-dried before being finely ground and incorporated into general-purpose diet (GPD) (Singh 1983) at the rate of 20% of the dry components. Plastic test tubes (5 ml) were approximately half-filled with the diet and frozen until required.

The test tubes containing the diet were inoculated with a single neonate BHLR larva on 9 May 2005, and held at 18-20°C under natural light. There were five replicates per treatment, with each replicate consisting of 25 inoculated test tubes. BHLR larvae reared on GPD were used as a control.

Larval mortality was recorded throughout the larval period, and pupation was assessed daily after 21 days. Percent larval survival, days to pupation, pupal sex and pupal weight at pupation were recorded.

Binomial generalized linear models were used to compare larval survival on the different diets. Larval development was compared by ANOVA of the log transformed number of days to pupation. Pupal weights were compared using ANOVA. Male and female pupae were analysed separately as the females are heavier than males (Clare & Singh 1988).

Bioassay 2 – leaf discs

Ten leaves were collected from each of the five Hort16A and five Hayward vines on 11 November 2004. Two discs, one 60 mm diameter and one 20 mm diameter, were cut from each leaf and placed on top of each other in a 150 mm Petri dish containing a moistened filter paper. Each Petri dish was inoculated with a single neonate BHLR larva, sealed with Parafilm® and held at 18-20°C under natural daylight. Leaf discs were changed weekly until the larvae neared pupation, at which time leaf discs were changed every 2 or 3 days as required. Fresh leaves were collected each time the leaf discs were changed.

Percent larval survival, days to pupation, pupal sex and pupal weight were recorded. Larval survival after 7 days and 21 days was analysed using Student's t-test.

Bioassay 3 – field cages

On each of the five Hayward and five Hort16A vines, 12 leaves and 12 fruit were selected and tagged without conscious bias when the leaves were approximately 8 weeks old and the fruit approximately 5-6 weeks old, that is, during the critical damage period. Actual dates were as follows: Hort16A leaves tagged 15 November 2004; Hayward leaves tagged 22 November 2004; Hort16A fruit tagged 12 December 2004; and Hayward fruit tagged 10 January 2005. Bud-burst occurs approximately 2-4 weeks earlier and fruit set approximately 4 weeks earlier on Hort16A vines compared to Hayward vines, hence the differences in the actual dates.

On the same dates, tagged leaves and fruit were inoculated with BHLR as follows. Three neonate BHLR larvae were placed inside a 30 mm long plastic straw. The straw was sealed with cotton wool at each end, attached to a tagged leaf or fruit stalk using blu-tac®, and the leaf or fruit covered with a muslin bag. Just before the muslin bag was sealed with a twisty-tie, the cotton wool at one end of the straw was removed to release

the larvae. Muslin bags were removed on 10 March 2005. Damage to caged leaves and fruit was recorded and graded according to severity using the following categories: 0=no damage, 1=negligible damage with a few surface bites only, 2=minor surface damage covering an area <1 cm^2, 3=moderate surface damage covering an area 1–2 cm^2 and 4=severe surface damage and any penetrating damage covering an area >2 cm^2.

The generalized linear mixed-effects model (GLMM) procedure was used to analyse the difference between the kiwifruit cultivars in the number of leaves or fruit damaged by larvae. The model used a binomial family with the penalized quasi-likelihood method, which is the superior method for clustered binary observations (Heo & Leon 2005). The severity of damage was analysed using the Kolmogorov-Smirnov non-parametric test on the recorded severity ratings. The method uses the largest difference between the two cumulative distributions of the ratings. All statistical procedures were carried out using R (R development Core Team 2005).

RESULTS

Bioassay 1 – artificial diets

BHLR survival was high on GPD with 82.4% of larvae surviving to pupation. Incorporating leaves into the diet had no effect on survival, with 83.2% and 85.6% of the larvae surviving to pupation on the Hayward and Hort16A leaf diets respectively. Larval survival was significantly lower (P=0.013) on the Hayward fruit diet (68.8%) than on the GPD control, but there was no difference in survival between the Hayward and Hort16A fruit (74.4%) diets.

Larvae reared on GPD developed through to pupation significantly faster (33.2 days) than those reared on the plant diets (Fig. 1). Larvae reared on diet containing Hayward or Hort16A leaves had almost identical mean developmental times (36.8 days and 36.2 days respectively), as did larvae reared on diet containing Hayward or Hort16A fruit (37.5 days and 38.0 days respectively).

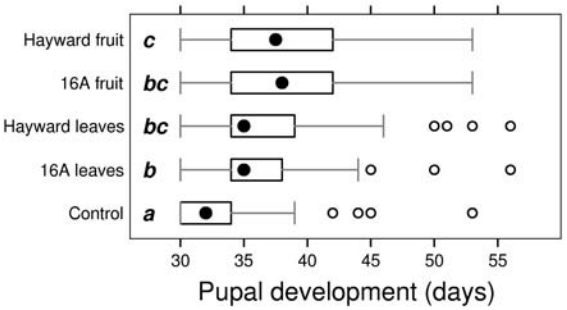

FIGURE 1: Box plots of development time for BHLR reared on diets containing Hayward or Hort16A plant material. Letters in bold inside the plot area are groupings at P=0.05 using Tukey's HSD on log transformed data.

Pupae from GPD were also significantly heavier (males=46.5 mg, females=60.8 mg) than those reared on the plant diets, with the exception of male pupae from the Hayward leaf diet (Fig. 2). There were no differences between the plant diets, with mean pupal weight ranging from 40.8 to 43.7 mg for males and from 50.5 to 54.9 mg for females.

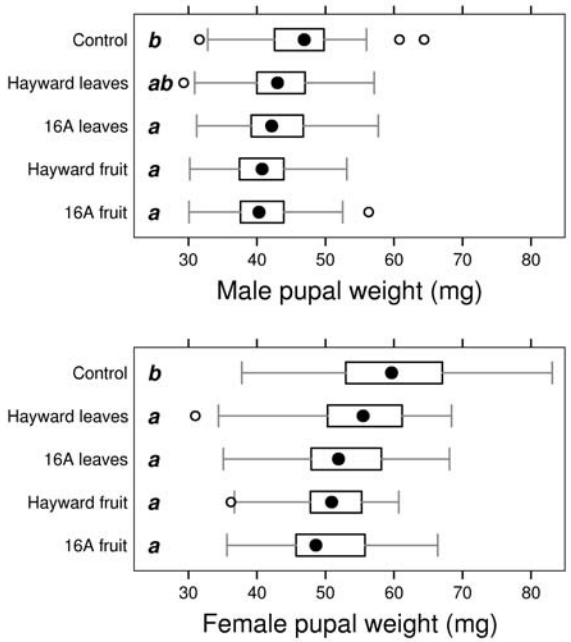

FIGURE 2: Box plots of pupal weights for BHLR reared on diets containing Hayward or Hort16A plant material. Letters in bold inside the plot area are groupings at P=0.05 using Tukey's HSD on untransformed data. Male and female data were analysed separately.

Bioassay 2 – leaf discs

Larval establishment was similar on the two kiwifruit cultivars as shown by the mean (±SEM) larval survival 7 days after inoculation, 86%±4.0 on Hayward and 78%±4.9 on Hort16A leaf discs (P=0.24). Larvae that died within 7 days of inoculation showed no signs of having settled and there was no feeding damage or frass on the leaf discs, which suggests this early larval mortality was most likely due to handling injury.

During the next 14 days few larvae died on the Hayward leaf discs and by day 21 most of the surviving larvae were 3rd instars. In contrast, there was considerable larval mortality on Hort16A. Larvae that died on Hort16A leaf discs during the 7 to 21 day period after inoculation had settled and fed on the leaf discs, but their growth and development were very slow and most were 1st or 2nd instars at the time of death. The difference between the mean larval survival on Hayward (74%±7.5) versus Hort16A leaf discs (10%±2.5) 21 days after inoculation was highly significant (P<0.001).

Only 16 larvae (32%) on Hayward leaf discs and three larvae (6%) on Hort16A leaf discs survived to pupation. The mean time to pupation and the mean pupal weight for larvae reared on Hayward leaf discs was 38.2 days and 18.6 mg respectively. Corresponding data for Hort16A leaf discs were 57 days and 14.8 mg. These data were not analysed because of the very low numbers of pupae. The reasons for the reduced survival on Hayward after 21 days are unknown.

Bioassay 3 – field cages

No larval cadavers or pupae were found on any of the caged leaves or fruit, but a considerable amount of larval feeding damage was recorded. This suggests that some of the released larvae survived, but they either died at an early instar (on leaves or fruit

with nil or minor feeding damage) or they escaped as a late instar through the seal on the bag (on leaves or fruit with severe damage). Leafroller larvae on kiwifruit vines generally pupate away from their feeding site (Steven 1990), and this behavioural factor may have triggered escape as a late instar.

Over three-quarters of the caged Hayward leaves were damaged (mean/vine=76.7%), and this was significantly greater (P<0.001) than for Hort16A leaves (mean/vine=35.0%). Damage on Hayward leaves was often in the moderate or severe category (mean damage score=2.7) and this was greater (P=0.013) than for Hort16A leaves where the damage was mostly negligible or minor only (mean damage score=1.8).

The mean percentage of caged Hayward or Hort16A fruit with larval feeding damage (53.0% and 70.0% respectively) was not significantly different (P=0.061). However, there were marked differences in the site and severity of damage. On Hort16A feeding damage to the actual fruit tended to be restricted to the sepal area, and usually consisted of a few surface bites covering an area less than 1 cm^2. However, there was often considerable feeding damage to the stalks of Hort16A suggesting that the larvae found the fruit unpalatable. The reverse was true on Hayward, with all damage occurring to the actual fruit including the sepal area, the side of fruit and the stamen area. The difference between the damage severity on Hayward fruit (mean damage score=4.0) versus Hort16A fruit (mean damage score=2.5) was highly significant (P<0.001).

DISCUSSION

Previous studies have shown that both Hayward and Hort16A are susceptible to attack by BHLR, but the incidence and severity of damage to Hayward can be twice that of Hort16A (McKenna et al. 1998*)*. This difference in susceptibility to BHLR was detected with the leaf disc and caging bioassay techniques, but not with the artificial diet bioassay technique. This suggests that measuring larval survival after 21 days on leaf discs, or the incidence and severity of damage to caged leaves or fruit, are both potential techniques for screening *Actinidia* plant material for resistance to leafrollers. However, it is important to consider other factors.

The main advantages of the leaf disc bioassay technique are its simplicity and the short period (3 weeks) required to achieve results. Early larval mortality due to handling was higher than is desirable, but this could be rectified by inoculating two neonate larvae onto each leaf disc and reducing the number to one after 3 or 4 days. Leaves were the only plant part tested in the Petri dish bioassays. However, leafroller larvae respond differently to different *Actinidia* plant parts. For example, BLLR can cause extensive damage to Hayward fruit, but is rarely found on the leaves (Steven 1990, 1991). Conversely, GHLR is often found feeding on the leaves of Hayward vines, but is rarely found on fruit. For this reason an equivalent disc assay for fruit will be required.

The main advantages of the caging technique are that it used intact plant material and it can be used on both leaves and fruit. However, because no larvae or pupae were present when the cages were removed, the number of released larvae that survived per leaf or fruit could not be determined. This is a major limiting factor of this technique and affects the interpretation of the results. For example, if two of the three released larvae survived the damage is likely to have been greater than if only one larva had survived. Leaving the bags on for a shorter period, and assessing the number and developmental age of larvae in addition to plant damage could potentially overcome this issue. Further work is required to develop this technique before the results can be used to assess differences between kiwifruit cultivars.

Results from the artificial diet technique were not promising with no difference in larval development and survival detected between Hayward and Hort16A. This technique can readily be used on different plant parts and, because the plant material or diets can be frozen, there is a high degree of flexibility in the timing of tests. It may be possible to achieve more discriminating results by increasing the proportion of plant material in the artificial diet. However, the plant material must be excised and processed and this may affect the results. For example, toxins or antifeedants present in the plant material

may be removed or diluted during the processing of the diets. Alternatively, if plant resistance is due to an induced response or a physical attribute of the plant then it will not be detected using artificial diets.

During the course of this study two techniques with potential for screening *Actinidia* for relative resistance to leafollers have been identified. Validation of these techniques is now required. This process should include experiments to determine whether consistent results can be achieved between experiments, and whether differences known from field observations between plant material of different ages, different leafroller species and different *Actinidia* species can be detected using the bioassay techniques.

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