A Method to Screen Pome Fruit for Resistance to Skin-chewing Insects and to Analyze Feeding Trends

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Abstract. Insect feeding traces on fruit are a major concern to orchardists. Breeding fruit for insect resistance is becoming more important as available pesticides are limited by more stringent regulations, problems of insect resistance, and residue limits. We present a method to analyze fruit skin damage via treatment of video images. This aspect has not been well studied to date, but would allow a more rapid assessment of fruit resistance to insects in breeding programs. The method uses equipment available on the world video and computer markets. Over 24 hours, larvae of lightbrown apple moth [Epiphyas postvittana Walker (Lepidoptera: Tortricidae)] were permitted to chew restricted areas of skin on the pear cultivars Sensation Red Bartlett, Packham’s Triumph, Doyenne du Comice, Beurre d’Anjou, and Corella. These authors considered it to be a more horizontal resistance (Dent, 1991; Wiseman, 1994). Breeding for natural or engineered plants that are resistant to pest damage is increasing (smith and Quisenberry, 1994). Breeding for natural resistance is rarely incorporated early, if at all.

Additional index words. apple, pear, Rosaceae, Malus ×domestica, Pyrus communis, Pyrus pyrifolia, Pyrus ×bretschneideri, lightbrown apple moth, Epiphyas postvittana, plant–insect, insect–plant interactions

Increasing restrictions on the use of chemicals in agriculture are leading to the development of integrated pest management. Therefore, interest in breeding and selection for natural or engineered plants that are resistant to pest damage is increasing (Smith and Quisenberry, 1994). Breeding for natural resistance is rarely incorporated early, if at all.
After this period, the larvae were removed and the fruit was stored for a maximum of 1 month at 0 °C in plastic bags to prevent desiccation until further analysis.

Video imaging. On the day of video imaging, fruit were removed from cold storage and left at ambient temperature for ≈10 min. Excess water from condensation was wiped with a tissue. The chewed areas that were still wet were then coated with talc powder (Fig. 1c) 30 min prior to video imaging and allowed to dry. This process increased the contrast on the black and white image. In the second year, the talc was not employed, as the chewed area on most hybrids rapidly darkened and presented sufficient contrast to be recognized by the software. The few damaged areas not dark enough were blackened with a marker. In 95% of the samples, the larvae fed only on the skin. Some larvae burrowed into the fruit flesh slightly (2–5 mm). However, this feeding was not measured, because only the area was detectable on the video image.

Images of fruit were taken with a video camera (model TK C1381EG; JVC, Tokyo) equipped with a zoom lens (Cosmicar ES 12.5–75 mm, Englewood, Colo.). The camera was connected to a video monitor, which was linked to a computer equipped with the frame grabber Targa+ (TrueVision, Santa Clara, Calif.). Some frame grabbers do not require the use of the extra video monitor. The images were saved as 8-bit grey scale image files, using the SigmaScanPro image measurement software (SPSS, Chicago). This software is one of many that allows the measurement of an area by flooding a zone of defined contrast. All fruit were placed 1 m from the camera to allow comparison. For area recognition, the grey intensity threshold delimiting the edges of the wound can be read on the computer screen by moving the cursor across the edges. The threshold can then be set so that the area marked by the software exactly matches the wounded area. The measurements were then achieved by clicking once on wound areas, and the area values were automatically reported in an attached table. The total area on each fruit available to one larva was estimated by inking the top edge of the vial and pressing it to the fruit skin. The area determined by the video-computer system was initially determined in square pixels. To express the results in metric units, calibration was obtained by comparing the mean of 10 total areas = 24,505 ± 433 pixel² (mean ± SE) to the known area of the vial opening (154 mm²).

Data analysis. Feeding ability was analyzed in two steps. The total area fed upon and the number of wounds per fruit were recorded only for fruit on which feeding was observed. The area/wound was then calculated. The experimental unit was the individual fruit. In the cultivar comparison analyses for ‘Beurre d’Anjou’ and ‘Corella’, data obtained on green and red sides were analyzed separately to compare the effect of color on feeding. The results were analyzed using analysis of variance and Kolmogorov-Smirnov normality tests (SigmaStat, version 2, SPSS). Multiple comparison analyses were made using Tukey’s test. Fruit with missing larvae or larvae that pupated were not considered in the analyses.

Results and Discussion

Practicality of the method. Assessing the insect resistance of the fruit of 75 trees can be achieved in a week (on the basis of 10 fruit/tree and 30 h/week). This can be divided into three steps: 1) selection of larvae, 2) infestation, and 3) image capture and analysis. Selecting the larvae and placing in individual vials can be achieved at a rate of 50 larvae/h. The vials should be closed temporarily to prevent the escape of larvae. The vials can be set on fruit at a rate of 100 fruit/h. The images can be scanned and the chewed area computed at a rate of 100 images/h. These estimates were determined...
during the second year trial. The major limiting factor is the size of the insect colony, which needs to be adjusted to the experimental design to be able to find the required number of larvae of the required instar at one time.

The method was developed with harvested fruit, but could easily be adapted for fruit on the tree. Once the larvae are selected, they can be brought to the orchard where infestation can probably be achieved at the rate cited above. If field applications are considered, vials with aeration holes should be used to avoid plant volatile accumulation and water condensation. The vials could be placed on the shady side of the fruit or tree to avoid overheating. Image capture can at present be achieved with small digital cameras. The risk of introducing large numbers of pests in the orchard is limited, as there were only four missing larvae out of 670 larvae set onto fruit within the 24-h experiment.

The nature of the vials (e.g., polycarbonate) may induce some volatiles to be released in the headspace, which may influence feeding behavior of the insects. This fact restricts the use of this method to comparisons between treatments or with a control assayed in an identical manner.

**Commercial cultivars.** Cultivars differed in the total amount of skin chewed (Fig. 2a). Larvae fed up to 3.4 times more on 'Sensation' than on 'Ya Li' pears. These two cultivars have an obvious visual difference in that the skin of 'Sensation' was mostly red, whereas that of 'Ya Li' was all green. However, the difference in feeding is unlikely to be due to differing pigments, because feeding was not significantly different between the green and red sides of 'Beurre d’Anjou' and 'Corella'.

The causes of differences in feeding ability are numerous and have been studied on many crops in relation to various phytophagous insect larvae. Among these causes, studies have highlighted the effects of compounds that either render the plant less digestible, e.g., cellulose or lignin (Hochuli, 1996), or more attractive, e.g., surface nutrients (Städdler, 1986). All of these compounds could influence the amounts of skin chewed. Differences in feeding behavior cannot be caused only by a difference in skin color, the red color in pome fruit skin being due to a complex blend of anthocyanins, flavonols, and other compounds (Lancaster, 1992). The difference in feeding on 'Sensation' and 'Ya Li' may also be related to the time difference between bloom and harvest, that is, to differences in fruit maturity, but this was not tested in this experiment. If fruit maturity is important, integrated pest management practices may be influenced (i.e., fewer insecticide treatments may be needed as fruit approach maturity). In any case, DAFB and other measures of maturity should be critical parameters in further development of this method.

In addition to the total area chewed, the image analysis system allowed us to count the number of wounds made by each larva. The mean surface area of each wound (Fig. 2b) was the result of higher numbers of smaller wounds than on 'Sensation'. This may reflect some properties of 'Comice' skin that prevented continuous feeding, such as compounds that cause a rejection response, as observed in other lepidopterous larvae (Glendinning, 1996). Some chemical components of the cuticle or skin of 'Ya Li' pears may have reduced the larval feeding time, relative to that on 'Comice', as total area chewed was smaller (Fig. 2a) despite similar area/wound (Fig. 2b). Time studies over the feeding period could determine whether large wounds result from continuous feeding or from small wounds joined together by more frequent feeding episodes.

The above results did not take into account the cases in which larvae did not feed at all. The absence of feeding ranged from 42% for the red side of 'Corella' to 10% for 'Twentieth Century'. The mean among all cultivars was 22% nonwounded fruit. The only significant difference (P ≤ 0.05) was between the red side of 'Corella' and 'Twentieth Century'. Measuring the absence of feeding may be worthwhile in breeding programs, but the method described here may not be suitable. The use of the vial to restrict the larva to the fruit prevents it from choosing between the fruit and other parts of the plant, a choice that is important in larval establishment (Suckling and Ioriatti, 1996).

**Hybrid seedlings.** The frequency distribution of the feeding area on fruit of 67 hybrid lines was graphed (Fig. 3). The normality test failed, with a K-S distance = 0.228 and P = 0.002. Figure 3 illustrates the wide variation in insect resistance that can be obtained in the first generation. There were three noticeable groups of hybrids. For one group (four hybrids) damage was ≤ 20 mm 2 , revealing some natural resistance in the fruit. For a second group (60 hybrids) damage averaged 40–45 mm 2 , the median sensitivity of most hybrids, and for a third group (three hybrids) it exceeded 100 mm 2 , revealing high sensitivity to larval feeding. The variations in feeding ability by the larvae seemed related neither to the size of the fruit, as there were both large or small fruit, with diameters ranging from 3 to 5 cm among both resistant and sensitive hybrids, nor to the color of the skin, as all fruit were green except for one resistant hybrid. The skin of its fruit was totally russeted, and probably provided some mechanical and/or chemical defense (Faust and Shear, 1972).

The above results take into account only fruit on which larvae fed. Nonwounded fruit varied from 0% to 70% among all hybrids, with a mean of 38%, but the correlation between area fed and absence of feeding was weak, with a logical tendency for small area being related to high absence of feeding (data not shown).

![Fig. 2.](image-url) (a) Mean area consumed by one light brown apple moth larva in 24 h on one fruit of each of seven commercial pear cultivars; (b) mean area per wound. Mean separation by Tukey’s test (P ≤ 0.05). Means are based upon 30–50 fruit per cultivar. The adjective “red” or “green” are for data collected on red or green sides of ‘Corella’ or ‘Beurre d’Anjou’ fruit.
Conclusion

The method presented here offers possibilities to quantify the amount of insect damage to fruit skin, an aspect that has not been well studied. This method uses standard, readily available video equipment and software. It would allow the screening of hybrids at an early stage of breeding (with an average rate of 75 per week).

The fruit skin of the *Pyrus* species apparently possesses some mechanism of resistance to larval feeding, as could be expected, given the broad range of skin textures and colors in this genus (Hummer, 1989). The resistance developed by the skin can probably be ascribed to a complex range of plant defenses (Schoonhoven, 1996). The different feeding trends described in this paper suggest that pears possess various mechanical and/or chemical defenses leading to smaller wounds by insect larvae. Also, breeding for deterrent effects may seem more advantageous, as they may result in smaller amounts of wounded fruit. However, all fruit properties that limit insect feeding ability may result in lower population densities, which would improve crop quality over a whole season.

In natural situations, the adaptation of insects to plant defenses through feeding exposure (Lindroth and Weisbrord, 1991; Papaj and Prokopy, 1989) may result in increased larval feeding. This could be checked by varying the rearing medium (i.e., leaves or fruitlets) before starting the feeding test. Other factors, such as attraction to volatiles and nesting possibilities, may also influence damage to fruit skin (Suckling and Ioriatti, 1996). However, the larval feeding ability per se remains a critical factor. Under natural conditions, when the fruit skin is not appreciated by the larvae, they tend to feed on leaves only. In our experiment, because of the absence of choice, the larvae may have fed on fruit, whereas in the orchard they would not have. Thus, we may slightly underestimate the natural resistance of a fruit by this method.

Literature Cited


