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An Automated System for Counting Achenes on Strawberries

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Development of strawberry (*Fragaria ×ananassa* Duch.) fruit depends on the number of fertilized achenes on its surface (Nitsch, 1950). The achenes are distributed on the receptacle surface in a pattern of more or less regular rows, spirally arranged (Takeda et al., 1990). The total number of achenes per fruit is determined during flower bud initiation and depends on fruit hierarchy on the inflorescence, cultivar, and environmental factors (Webb et al., 1978). A quadratic relationship exists between fruit size and fertilized achenes (Khanizadeh et al., 1993).

The total number of achenes per fruit is of great importance to strawberry breeders. The number of achenes may be determined by 1) counting the number per square centimeter of surface on ripe fruit (Abbott et al., 1970; Abbott and Webb, 1970); 2) weighing the achenes after separation from the receptacle; or 3) counting the number of achenes after pressing the fruit between two layers of glass (Khanizadeh, 1983). These methods are laborious and time-consuming.

Takeda et al. (1990) estimated total achene counts using a regression line based on the number of carpels in a spiral. However, this method only can be used to study the relationship between fruit weight and achene count if all the carpels are fertilized and may not be suitable for malformed fruit that are partially pollinated and may have many aborted achenes.

We, therefore, tested a new method as an alternative to the methods noted. An image analysis system (IAS) was developed by Vigneault et al. (1992a) for measuring the mean diameter of air bubbles in a water tank. This system consists of a monochrome camera that produces a video signal. The frame grabber in the PC digitizes the image in a 480 × 512 pixel matrix with eight bits of resolution per pixel in real time using an Occulus -300 board (Coreco, St. Laurent, Quebec) installed on an IBM-AT compatible microcomputer. The background light designed previously for this instrument (Vigneault et al., 1992b) was modified by adding a red filter to discriminate the achenes from the liquid. The software was modified to identify and count the achenes in the viewing field of the camera. The IAS separates the achenes into two groups: aborted (smaller) and sound (larger), based on their projected surface area.

'Kent' and 'Glooscap' were used to test the accuracy and time required by this system to estimate the sound and aborted achenes compared to manual counts. About 150 fruit per cultivar were selected from primary, secondary, tertiary, and quarternary inflorescence positions. Fully pollinated and malformed fruit ranging from 3 to 20 g were collected and kept in a freezer (–20C) until needed.

Two procedures were used for preparing the samples before counting the achenes: 1) normal fruit (having few aborted achenes) were cut in half lengthwise and pressed between two layers of glass to distribute the achenes as described by Khanizadeh (1983); and 2) severely malformed fruit were commutated in a mini blender (2 min), and the pulp was then pressed between two layers of glass. The aborted and sound achenes were manually counted, and then the samples were transferred to the IAS to estimate the number of sound and aborted achenes.

An F test was used to evaluate the differences between the two methods after removing the effects of fruit size, fruit hierarchy, and harvest time. The accuracy of the IAS was evaluated by comparing the number of sound and aborted achenes with those obtained by manual counts. Count data were transformed using an arcsin square root percent transformation (Steel and Torrie, 1980) before analysis of variance (ANOVA). The ANOVA was done using the general linear model procedure (GLM) of SAS (SAS Institute, Cary, N.C.).

No significant difference was observed between the two methods with respect to the total number of sound (≈140) or aborted achenes (≈50) per fruit, indicating that the accuracy of the IAS is similar to that of the manual counts. The time required to estimate achenes per fruit optically (2.85 min) averaged significantly less (20%) than manual counting (3.54 min).

A wide range of variation in achene colors occurs among strawberry genotypes (Khanizadeh et al., 1994). The IAS was most accurate with darker achenes (black, brown, green) on a light flesh background. Adjustment of light intensity and sensitivity was required for lighter-colored achenes (honey color, white, and yellow) on a light flesh background. This problem can be solved by removing some of the flesh or adding water before commutating the strawberry in the mini blender or by readjusting the light intensity of the instrument. To our knowledge, however, no solution is available for very light flesh with light-colored achenes, since the IAS is not capable of detecting the yellow or honey-colored achenes on a light background (i.e., unripe fruit with yellow or white flesh). In this case, dyeing the achenes before counting might solve the problem. The IAS cannot differentiate between single or closely grouped achenes. Therefore, an even distribution of achenes results in greater accuracy. The method described here facilitates the study of the relationship between fruit weight and number of sound achenes and improves achene counting efficiency by reducing eye fatigue and human error. The IAS also is capable of grouping the achenes into two or more categories based on color and size.

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Literature Cited

Abbott, A.J., G.R. Best, and R.A. Webb. 1970. The relation of achene number to berry weight in strawberry fruit. J. Hort. Sci. 45:215–222.

Abbott, A.J. and R.A. Webb. 1970. Achenes spacing of strawberries as an aid to calculating yield. Nature (London) 225:663–664.

Khanizadeh, S. 1983. The effect of two fungicides in stigma and pollen viability of three strawberry cultivars (*F. ×ananassa*) and the impact of these on fruit quality. M.S. Thesis, McGill Univ., Quebec.

Khanizadeh, S., J. Fortin, M.J. Lareau, and D. Buszard. 1994. Sensory evaluation of six strawberry cultivars after machine harvest. In: H. Schmidt and M. Kellerhals (eds.). Progress in temperate fruit breeding. Kluwer Academic Publishers, The Netherlands. (In press.)

Khanizadeh, S., M. Lareau, and D. Buszard. 1993. Effect of flower thinning on strawberry fruit size and its relationship to achene number. Acta Hort. 348:351–356.

Nitsch, J.P. 1950. Growth and development of the strawberry as related to auxin. Amer. J. Bot. 37:211–215.

Steel, R.G.D. and J.H. Torrie. 1980. Principles and procedures of statistics, a biometrical approach. 2nd ed. McGraw-Hill, New York.

Takeda, F., G.W. Lightner, and B.L. Upchurch. 1990. A rapid method of determining carpel numbers in strawberry flowers. HortScience 25:230.

Vigneault, C., B. Panneton, and G.S.V. Raghavan. 1992a. Image analysis of 3-D clouds of bubbles. Can. Agr. Eng. 34(4):347–352.

Vigneault, C., B. Panneton, and G.S.V. Raghavan. 1992b. Real time image digitizing system for measurement of air bubbles. Can. Agr. Eng. 34(2):151–155.

Webb, R.A., J.H. Terblanche, J.V. Purves, and M.G. Beech. 1978. Size factors in strawberry fruit. Scientia Hort. 9:347–356.