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Determination of Pollen Number of Beans Using an Electrical Particle-counting Device

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Abstract. **The number of pollen grains in anthers of *Phaseolus vulgaris* L. was estimated using a Coulter Counter, an electrical particle-counting device. Nine green buds were collected randomly from 3 plants grown in a growth chamber. From each bud, one anther at a time was excised and placed in liquid N for 3 sec. Pollen grains were transferred from the anthers into a drop of 0.3 M NaCl on a glass slide. The saline-pollen grain mixture was cleaned of all debris, and pollen was washed into a beaker containing the same solution. This procedure was repeated for the remaining 9 anthers of each bud. The total number of pollen grains per bud was determined using a Coulter Counter.**

A method of counting particles with a Coulter Counter was introduced over 25 years ago by Coulter Electronics, Inc. (Hialeah, FL 33010). Using this technique, particle suspended in an electrolyte can be counted by passing them through an aperture in which an electrical current flows. As particles pass, resistance in the path of the current changes: the number of recorded changes indicates the number of particles passing through the aperture. Thus, the total number of particles within the sample can be ascertained. This technique has been used primarily to count blood cells, but it can be adapted easily for counting pollen grains.

A number of techniques, most of which utilize a light microscope, are used presently to count pollen grains. Pollen is mounted on glass slides for counting, but because the number of grains is usually large, the common methods utilize subsamples (4). A relatively small number of pollen grains actually is counted, and the estimate of total number of grains is an extrapolation. Such a counting procedure is tedious, and the repetition often produces widely divergent estimates. A reliable pollen counting technique appears to be the haemacytometer method (5, 7, 8). This technique involves counting pollen grains suspended in a known volume of liquid on a glass slide that is divided into counting chambers.

Because determination of total pollen produced is important in hybridization studies and in research on reproductive biology and pollination mechanisms, it is important to establish a rapid, reliable method of assessing the total amount of pollen produced by a flower or by a plant.

Plants of 'Light Red Kidney' were grown in a growth chamber at a day/night temperature of 25°/19°C with 21.6 klx of light for 16 hr daily at 50% to 60% RH. Three plump green buds, in which anthers and pollen were considered to be mature (9), were excised from each of 3 plants at the onset of the day regime and were placed separately in labelled vials, which were immersed in an ice bath. Each bud in turn was examined under a dissecting microscope illuminated with an indirect light source. The floral keel was removed intact, and one anther was excised with a segment of the filament attached. The keel was returned to the ice bath. To reduce the tendency for pollen grains to adhere to each other or to the anther wall, the excised anther was placed in liquid N for 3 sec. It was submerged then in a drop of an electrolyte solution, 0.3 M NaCl, on a glass slide. Staining with Alexander's triple stain (1) indicated that liquid N treatment did not damage pollen.

The filament was manipulated with tweezers under a dissecting microscope so that the anther was oriented with the stomia facing upward. The stomia were opened gently, and pollen grains were transferred carefully into the saline solution using a 2nd pair of tweezers. Calgon or Tween 20 was used to reduce surface tension and improve pollen grain dispersal in the solution; pollen congregates could clog the Coulter Counter aperture. The anther was agitated gently in the solution to dislodge any remaining pollen grains, and it and all anther fragments were removed from the solution. The saline-pollen grain mixture was washed off the slide with additional 0.3 M NaCl into a beaker and placed in an ice bath. The entire procedure was repeated for the remaining 9 anthers of that bud and subsequently for the other 8 buds. The removal of pollen grains from the 10 anthers of a single bud took about 10 min.

The 0.3 M saline solution was appropriate for *P. vulgaris* pollen, since it was only slightly hypertonic in relation to the grains. However, pollen of other species probably would require different saline concentrations or electrolyte solutions. The instruction manual for Coulter Counter Model Z_B (6) suggests, for example, 4% sodium pyrophosphate or 0.25% NaCl in glycerine/water (75/25 percentage volume ratio).

The number of pollen grains in each sample was determined using a Coulter Counter model Z_B. The aperture diameter was 140μ based on the recommendation (6) that size of particles to be measured be 2% to 40% of the aperture diameter. Pollen grains from anthers of 3 buds of one plant were pooled for counting in one sample so that pollen count per 0.5 ml of saline solution was above 1000 but below 10,000 (6). A stirring rod was used to maintain uniform distribution of pollen grains within the saline solution, and grains were stained with cotton blue to ascertain visually the uniform dispersal.

Ten readings per sample were taken, with coefficient of variation of about 8%. Total number of pollen grains in each 3-bud sample from 3 different plants was 6576, 6834, and 7353.

Pollen count estimations using light microscope methodology range from 429 to 2140 for *P. vulgaris* (2, 4), but counts could diverge widely, based on the cultivar sampled, the bud size and position on the plant and the environmental conditions (2, 4, 5).

The green buds selected for this study were plump and firm, and anthers and pollen were regarded as mature (8). Extreme care was taken to ensure pollen integrity; however, Coulter Counter data indicated some variation in grain size. This variation suggested that some pollen was not mature or was not intact. Although the focus of this work was on counts rather than on measurements of size, this information is important in relation to bud selection and anther and pollen manipulation prior to counting. We suggest that in samples where significant pollen damage is evident, a control sample of a known pollen grain count, established microscopically, should be used to indicate lower and upper threshold limits for determining lower and upper grain size limits for the count. This information then would serve as a standard for testing samples from the same plant.

Pollen integrity during counting might be enhanced by fixation or critical point drying prior to immersion in an electrolyte solution. Gabrieli and Wertheimer (3) preserved ragweed pollen grains in 15% formalin in a saline solution. The major constraint if the Coulter Counter is to be used with fixed grains is that the fixative not interfere with the electric current flow through the aperture.

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It appears the Coulter Counter can be used satisfactorily to count pollen grains of *P. vulgaris*. Samples were analyzed quickly with adequate precision, without the need of subsampling. The total time for harvest and count of 9 buds of *P. vulgaris* for pollen count was about 2 hr, of which 1.5 hr were spent removing pollen from anthers. The Coulter Counter can be used to obtain data on change of number of pollen grains under various environmental conditions as well as to analyze pollen distribution within a plant or within a plant population. The potential limiting factor in the present experimental procedure was maintenance of pollen integrity: it was ensured by use of proper electrolyte media, careful handling of pollen during extraction, and rapid analysis. The Counter Counter technique should be a useful addition to the existing pollen counting methods.

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NOTES

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Automatic Irrigation in a Peach Orchard

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Systems to control water supply commonly use one or a combination of the following measurements: 1) soil-water suction using tensiometers, 2) soil-water content from the neutron moisture meter, 3) pan evaporation, 4) rate of fruit growth, or 5) timing based on the knowledge of soil, plants, and environment in a particular orchard (1, 3, 4, 5). Most of these methods require a regular commitment of time and management skill; some are expensive (neutron meter) or need constant maintenance (tensiometers). The following report describes a matric suction sensor (MS sensor) that controls irrigation through soil-water suction.

The MS sensor detects a predetermined suction in the root zone and switches water on and off accordingly. The sensor measures electrical conductivity within a porous ceramic block, 25 × 50 × 100 mm in size. The electrical conductivity is related to the water content of the porous block and, therefore, to the matric suction of the soil water with which it is in contact (2). The sensor simultaneously measures the electrical conductivity in a fine-pored powder packed into a cavity in the block. This conductivity depends upon the concentration of solutes in the soil water, and, when offset against the conductivity of the porous block, it compensates for the salinity of soil water. An electronic circuit built into the block generates a voltage which rises as the soil dries (sensing voltage) and a voltage related to salinity (compensating voltage). It continuously monitors the difference between sensing and compensating voltages. When this difference is positive, an external circuit turns on the water supply valves and turns them off when negative. Sensors have been produced (Irrigation Supplies, 2001 Third Street, Riverside, CA 92507) to switch at about 10 kPa, 15 kPa, and 25 kPa matric suction using special porous ceramics.

The sensors are soaked in water and then installed in augered holes 100 mm in diameter. A soil slurry is thoroughly worked around the sensor to establish hydraulic contact with the soil. The hole is backfilled and repacked firmly enough to eliminate large voids.

The 15 kPa MS sensor was buried 120 mm deep in a 6 liter pot containing 2 corn plants (*Zea mays* L. 'XL 66'), together with a tensiometer fitted with a pressure transducer. The sensor switched on and irrigation started 6.7 hr after monitoring began, when the sensing voltage equalled the compensating voltage; soil suction was then 17.2 kPa (Fig. 1). Watering reduced soil suction over the next 2 hr, and the sensor switched off again at 8.4 hr. The cycle was repeated at 27 hr, switching on at 14.8 kPa. This sensor irrigated through 5 cycles of drying and wetting, switching on at mean suction 15.7 ± 1.1 kPa. The same sensor was stable in saline soil, switching on at 14.9 ± 0.8 kPa in soil having EC (1:5 soil suspension) equal to

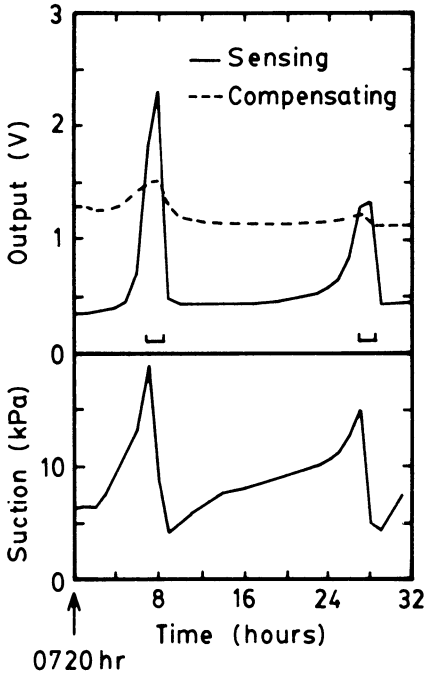

Fig. 1. Output from the matric suction sensor, and soil-water suction, during 2 cycles of switching in a pot containing 2 corn plants. □ = irrigations.

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