BARD

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PROJECT NO. US-1996-91

**Electrostatic Processes Underlying Natural and
Mechanized Transfer of Pollen**

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I. ABSTRACT (Electrostatic Processes Underlying Natural and Mechanized Transfer of Pollen)

The project objective was to more fully understand how the motion of pollen grains may be controlled by electrostatic forces, and to develop a reliable mechanized pollination system based upon sound electrostatic and aerodynamic principles. Theoretical and experimental analyses and computer simulation methods which investigated electrostatic aspects of natural pollen transfer by insects found that: a) actively flying honeybees accumulate ~ 23 pC average charge (93 pC max.) which elevates their bodies to ~ 47 V likely by triboelectrification, inducing ~ 10 fC of opposite charge onto nearby pollen grains, and overcoming their typically 0.3-3.9 nN detachment force resulting in non-contact electrostatic pollen transfer across a $5\text{ }\mu\text{m}$ or greater air gap from anther-to-bee, thus providing a theoretical basis for earlier experimental observations and "buzz pollination" events; b) charge-relaxation characteristics measured for flower structural components (*viz.*, 3 ns and 25 ns time constants, respectively, for the stigma-style vs. waxy petal surfaces) ensure them to be electrically appropriate targets for electrodeposition of charged pollen grains but not differing sufficiently to facilitate electrodynamic focusing onto the stigma; c) conventional electrostatic focusing beneficially concentrates pollen-deposition electric fields onto the pistil tip by 3-fold as compared to that onto underlying flower structures; and d) pollen viability is adequately maintained following exposure to particulate charging/management fields exceeding 2 MV/m.

Laboratory- and field-scale processes/prototype machines for electrostatic application of pollen were successfully developed to dispense pollen in both a dry-powder phase and in a liquid-carried phase utilizing corona, triboelectric, and induction particulate-charging methods; pollen-charge levels attained (~ 1 -10 mC/kg) provide pollen-deposition forces 10-, 77-, and 100-fold greater than gravity, respectively, for such charged pollen grains subjected to a 1 kV/cm electric field. Lab and field evaluations have documented charged vs. uncharged pollen deposition to be significantly ($\alpha = 0.01$ -0.05) increased by 3.9-5.6 times. Orchard trials showed initial fruit set on branches individually treated with electrostatically applied pollen to typically increase up to ~ 2 -fold vs. uncharged pollen applications; however, whole-tree applications have not significantly shown similar levels of benefit and corrective measures continue. Project results thus contribute important basic knowledge and applied electrostatics technology which will provide agriculture with alternative/supplemental mechanized pollination systems as traditional pollen-transfer vectors are further endangered by natural and man-made factors.

הספריה המרכזית
למדעי החקלאות
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II. OBJECTIVES

II.A. BACKGROUND AND RATIONALE

Successful crop production in orchards, greenhouses and the field depends upon the degree of plant pollination achieved, especially for plants where the fruit or seed is the harvested crop. Angiosperm plants are typically pollinated in three sequential mass-transfer phases: a) release of pollen from the "male organs"--the anthers; b) conveyance of pollen by biotic or abiotic carriers; and c) placement of pollen on "female organs"--the recipient surfaces of the stigmata.

Natural pollen transfer is achieved by a number of vectors, of which the most important are wind, animals--usually insects, and bio-mechanisms which deposit pollen onto the stigma of the same flower. The importance of vectors depends on the breeding system of the plant in question. Although some plants are self-compatible and set viable seed to their own pollen, many species are self-incompatible with a genetic system for rejection of self pollen by the female tissues of the flower (*e.g.*, alfalfa, cabbage, cauliflower, almonds, and many stone and pome fruits). Some species are self-compatible but monocious, with separate male and female flowers on different parts of the plant (*e.g.*, maize, cucurbits). A few such as the date palm and kiwifruit are dioecious with separate male and female plants. Although the importance of vectors in achieving pollen transfer between different flowers is obvious for self-incompatible, monocious and dioecious plants, it is also crucial for many of the self-compatible species (*e.g.*, tomato) that lack mechanical self-pollination devices and for which optimum fruit set depends on the presence of insects to move pollen from anthers to stigma within or among flowers. Where breeders have incorporated male sterility into parent lines for production of heterotic hybrid seed, pollen vectors become more important as pollen must be moved from the male-fertile individuals to the male-sterile "female" parents from which the commercial hybrid seed is harvested.

Most wind-pollinated crops present no pollination problems so long as favorable weather occurs during flowering. There is potential, however, for mechanical enhancement, particularly for dioecious wind-pollinated species such as the date palm where only the female trees produce a crop and their numbers need to be maximized relative to interplanted male trees. In contrast to wind-pollinated species, insect-pollinated crops often show severely limited levels of pollination, especially in intensive horticultural regions where heavy use of pesticides has decimated indigenous insect populations. Cultivated plants which have been developed through selective breeding can

differ greatly in both shape and character from their native ancestors exhibiting flowers no longer in accordance with their original pollinating agents. In addition, many species have been geographically separated from their co-adapted natural pollinators. Thus, cultivated plants may suffer from lack of pollination in their new artificial habitat as is the case for tomatoes, originally pollinated by local solitary bees in South America; for almonds, originally pollinated by local solitary bees in the Middle East; and for avocado, originated in Central and South America and the West Indies, and pollinated probably by stingless bees.

Past efforts to overcome low pollen-transfer efficiency have centered mainly on domesticating special biotic pollinating agents. Widely used ones are *megachile* bees, *alkali* bees for alfalfa, *Osmia* for apples and plums, bumblebees for kiwifruit, clovers and tomatoes, and *Peponapis* and *Xenoglossa* for squash. There remain, however, a number of economically important crops in the US and Israel for which neither honeybees nor these alternative pollinators are satisfactory for achieving a high level of pollination and subsequent yield. These crops include avocado, macadamia, pistachio, most cultivars of almonds and certain apples, kiwifruit, mango, papaya, canola, pecans and alfalfa. An improved physical means of pollination would help overcome this difficulty; it would concurrently facilitate a much needed reduction in resource input per unit of crop production by increasing the biological efficiency of individual plants, thus enhancing economic return and international competitiveness of US and Israeli agriculture.

Other problematic factors in achieving reliable crop pollination are tracheal mite infestations as well as movement of Africanized bees into significant agricultural regions of the US. Mites have caused major reductions in indigenous beneficial bee populations and further reductions could result directly from aggression of the Africanized bees as well as indirectly by deterrent pesticidal strategies aimed at halting the incursions.

There is thus a critical need for an efficient mechanized pollination method and its associated devices. Fortunately, highly developed technology now exists for electrostatic management of the motion of small particulates the size of pollen. Based upon this technology are numerous commercial and industrial processes including xerographic photocopying, laser ink-jet printing, electrostatic powder coating, electrostatic crop spraying, and electrostatic precipitation of air pollutants. Electrostatic forces are probably also naturally active during bee-pollination of certain

flowers. Thus, the following **hypotheses** have guided this BARD research project toward helping alleviate pollination deficiencies and problems:

Hypothesis A - In plants with flowers adapted to insect pollination, electrostatic forces play an important role along with other mechanical forces in the detachment, transfer and reattachment of pollen in its movement from anther to insect and from insect to stigma.

Hypothesis B - Knowledge of electrostatic processes underlying natural pollen transfer applied in conjunction with newly available high-tech charged-particulate control means, such as the latest developments in xerography, ink-jet printing, and laser copiers, will enable the engineering design and development of an efficient system for the harvesting and focused deposition of pollen on a commercial scale for high-quality crop production.

II.B. ORIGINALLY PROPOSED OBJECTIVES AND RESEARCH PLAN

The **overall objective** of this work was to more fully understand how the motion of small pollen grains may be controlled by electrostatic forces, and to engineer a reliable commercial-scale mechanized pollination system based upon sound electrostatic and aerodynamic principles. **Specific objectives** were:

- a) To investigate the role of electrostatic forces in the natural processes of pollen transfer by insects.
- b) To appropriately incorporate aerodynamic and electrostatic forces in the engineering design and development of a system for the mechanized harvesting and subsequent focused application of pollen on a commercial crop-production scale.

The **research plan** for achieving these objectives was conducted to develop both the process and the prototype machines required for mechanized pollination based upon electrostatic/aerodynamic principles. The three following stages of research tasks were evenly divided on a binational basis to US and Israeli teams of engineers and botanists:

Stage 1 - Investigate the electrostatic aspects of floral biophysics underlying naturally occurring bee/pollen force interactions in order to establish a reliable basis for mechanized pollination.

- a) Measure the dielectric and charge-relaxation properties of flower components under a range of atmospheric electrical conditions.

b) Develop a general computer simulation model for flower/bee electrical interaction based upon solution of Laplace's equation for electric potential distribution.

c) Experimentally map the electric field established within a flower upon entry of a charged body utilizing miniaturized field-sensitive crystal/fiber-optic probes which facilitate electrically non-intrusive measurements.

d) Determine the onset level for corona discharge induced to flow from sharp-edged flower components by atmospheric and bee-imposed electric fields and ascertain any consequential effects upon pollen repulsion and flower injury.

e) Establish the relative significance of the roles played by electrophoresis, dielectrophoresis and aerodynamic drag forces for effecting pollen transfer within the flower.

f) For imposed electric fields, determine their intensity vs. duration characteristics which establish the threshold for possible effects on pollen viability and quality.

Stage 2 - Develop a laboratory-scale electrostatic/aerodynamic device for mechanized pollination.

a) Devise and evaluate an efficient pollen collection method.

i) Maximize pollen detachment from the dehiscent anther by application of vibratory energy *via* an oscillating electric field, acoustics, *etc.* Determine the natural frequency of the stamen components in order to optimize vibratory-energy transfer for pollen detachment.

ii) Determine the appropriate balance of aerodynamic suction and electrophoretic/dielectrophoretic forces of attraction needed to efficiently gather the detached airborne pollen.

iii) Devise effective means for separating pollen from the collection air *via* appropriate combination of mechanical filtration, cyclonic separation and/or electrostatic precipitation device of novel design.

iv) Incorporate successful methods from steps above into a laboratory-scale device and experimentally evaluate its pollen collection efficiency.

b) Devise and evaluate effective materials-handling means for the collected pollen.

i) Disperse stored pollen from bulk to airborne state by proper applications of fluidized bed, acoustic and/or other particulate-dispersal methods.

- ii) Apply traveling electric-field techniques to devise a miniaturized pollen conveyance and metering system.
- iii) Experimentally evaluate the performance and metering accuracy of the combined pollen-handling system.
- c) Devise and evaluate an efficient pollen deposition method.
 - i) Optimize pollen electrification by proper incorporation of contact charging, corona charging and/or triboelectric charging methods.
 - ii) Properly balance aerodynamic and applied electric forces for precise trajectory of charged pollen grains to inner flower regions.
 - iii) Apply concepts of electrostatically-focused particle deposition to concentrate pollen transfer upon the stigma region of the style. Utilize air-ion current to precharge waxy-dielectric-coated-flower parts in order to converge electric-field lines selectively upon the conductive stigma for reducing pollen wastage on non-target surfaces.
 - iv) Experimentally evaluate the pollen-deposition efficiency of the lab-scale system.
- d) Experimentally evaluate the biological performance of the overall laboratory-scale system on selected container-grown plants.

Stage 3 - Develop and evaluate field-scale, worker-carried, prototype machines for accomplishing mechanized pollination in two independent field operations--harvesting and application.

- a) Design and construct portable high-voltage electronic circuitry for oscillatory detachment and collection of pollen.
- b) Design and construct a rugged, portable pollen harvester incorporating the above circuitry along with the successful pollen-collection methods established in *Stage 2*.
- c) Design and construct portable high-voltage electronic circuitry for powering the particle-charging system, the applied trajectory-field system, and the traveling-field metering system.
- d) Design and construct a rugged, portable pollen-applicator incorporating the above circuitry along with the best pollen-handling and deposition methods of *Stage 2*.
- e) Field test the engineering performance and the biological efficacy of the pollen harvesting and application machines on small plots of selected plants of differing morphology.

Time Schedule and location for individual research tasks.

Responsibilities and Location	First Stage	Second Stage	Third Stage
Ag. Engr. Dept. U.G.A., U.S.A.	1.a, 1.c, 1.d 1.f	2.a.iii, 2.b.ii 2.b.iii, 2.c.i-iv	3.c, 3.d, 3.e
Ag. Engr. Inst. A.R.O., Israel	1.b, 1.d, 1.e	2.a.i-iv, 2.b.i 2.b.iii	3.a, 3.b, 3.e
Hort. Dept. U.G.A., U.S.A.	1.f	2.c.iv, 2.d	3.e
Botany Dept. T.A.U., Israel	1.e, 1.f	2.a.iv, 2.d	3.e

II.C. DEVIATIONS AND REVISIONS OF RESEARCH PLAN

Under *Stage 1* of the research plan all research tasks were addressed with the exception of #1.c - aborted due to unavailability of miniaturized electric-field-sensitive crystal/fiber-optic sensor originally promised by Surrey University. Tasks #1.a was conducted only at laboratory conditions due to untimely withdrawal of Ph.D. student.

Under *Stage 2* all research tasks were addressed with the exceptions of #2.b.ii - traveling electric-field metering and conveyance of pollen was evaluated in laboratory but miniaturization of its 3-phase high-voltage electronic circuitry proved impractical for orchard use, and #2.c.iii - aborted because theoretical model/computer simulation taught that while electrostatically focused deposition of charged pollen preferentially upon the stigma was a theoretically valid approach, the magnitude of the benefit would be negligible due to rapid charge-relaxation of flower components (μ s - ns time scale) *vis-a-vis* the relatively slow charged-spraying event (ms time scale). Under task #2.c.i - contact charging of dry pollen was replaced by induction spray charging of pollen suspensions *via* existing electrostatic crop-spraying technology once a biologically compatible carrier liquid was formulated.

Under *Stage 3* task #3.e - the small-plot field testing was expanded to large-scale almond orchard trials and, additionally, a botanical field study of factors influencing early season almond flower and fruit drop was included beyond the originally proposed research plan.

III. RESEARCH ACTIVITIES AND RESULTS

III.A. INTRODUCTION

This three-stage project was actively pursued binationally in order to: understand the role which electrostatic forces naturally exert in pollination and develop an engineering/botanical design rationale for incorporating such forces into mechanized supplemental-pollination processes; develop pollen-charging and electrostatic-deposition technologies for both dry pollen grains and liquid-carried pollen as based upon corona, induction, and triboelectric particulate-charging methods, and subsequently utilize to fabricate/test aerodynamic-electrostatic prototype machines for harvesting/applying pollen, and laboratory evaluate their effectiveness/efficiency for depositing viable pollen grains onto stigmatic surfaces; and finally to field evaluate enhancements in fruit-set/yield achieved by the various electrostatic pollen-application processes developed. The following section III.B presents the relevant data, results and discussions, as well as specific laboratory and field methodologies developed to accomplish project goals.

III.B. SCIENTIFIC STUDIES AND ENGINEERING DEVELOPMENTS

Incorporated here into the body of this BARD final report are project-related publications, along with other subsections, documenting the following research activities:

1. Understanding natural electrostatic processes in pollination
2. R&D of pollen charging, metering, spraying, and deposition methods and devices
3. Laboratory simulation and validation experiments
4. Methods for assessing the count and germinability of pollen electrostatically deposited onto stigmas
5. Field tests for pollinating orchard crops
6. Almond fruitlet-dropping study

IV. EVALUATION OF RESEARCH ACHIEVEMENTS

The *overall objective* as stated in the proposal to BARD for this collaborative research project was . . . “*to more fully understand how the motion of small pollen grains may be controlled by electrostatic forces, and to engineer a reliable commercial-scale mechanized pollination system based upon sound electrostatic and aerodynamic principles*”. This section evaluates the project’s research achievements in relation to this originally proposed objective and, more specifically, how the 3-stage research plan has accomplished the two originally proposed sub-objectives.

Theoretical and experimental analyses as well as computer simulation methods were successfully applied in the *first sub-objective* . . . “*to investigate the role of electrostatic forces in the natural processes of pollen transfer by insects*”. Electrostatic aspects of the biophysics underlying naturally occurring bee/pollen/flower charge accumulations and their resultant force interactions were elucidated by numerical quantification, taking into account their transient (*i.e.*, time-dependent) characteristics. Experiments showed the average charge accumulated on a honeybee, as measured following active “laboratory flight” through 30-70% R.H. air, to be 23 pC with a maximum value of 93 pC. Considering the bee as a uniformly charged isolated sphere of ~ 0.5 pF capacitance, the corresponding electric potential attained by the bee was determined to be ~ 47 V. No measurable charge accumulated on honeybees without active wing flapping, emphasizing the likely role of triboelectrification during flight and “buzz pollination” events. Further analysis indicated that nearby charged bodies, such as the honeybee, induce opposite charge from earth onto individual pollen grains clustered on a grounded stigma (*e.g.*, ~ 11 fC typically for lizianthus flowers) resulting in pollen detachment under action of the ~ 4 nN force of attraction. Non-contact electrostatic pollen transfer across a 5 μm or greater air gap from anther-to-bee was thus theoretically confirmed in support of earlier experimental observations. Furthermore, detachment-force data required for rational engineering design of electrostatic pollen-harvesting processes/machines were established under the steady-state conditions of these experiments.

Associated research efforts under the first sub-objective more fully determined the electrical characteristics of individual flower structural components and their capability to transfer electric charge (*i.e.*, their charge-relaxation time constant τ) in response to transient electric-field interactions arising from rapidly approaching charged bodies such as bees or charged pollen clouds entering the

flower. Numerical characterization of floral charge-relaxation successfully: a) confirmed it to be adequately rapid in the style-stigma structure to ensure real-life validity for the above steady-state pollen-detachment force study as well as ensuring stigmas to be electrically appropriate targets for electrodeposition of charged pollen grains; and b) guided possible exploitation of differential charge accumulation on slower charge-relaxing ($\tau = 25$ ns) waxy petal surfaces which might electrodynamically focus like-charged pollen grains onto the more rapidly relaxing ($\tau = 3$ ns) stigma style. Computational results of a 3-D finite element model, which was coupled with an instantaneously introduced $25 \mu\text{C}/\text{m}^3$ pollen-cloud space charge and transiently equilibrating boundary potentials on the distinct flower-component surfaces, indicated a 3- to 5-fold intensified electric field (up to $24 \text{ kV}/\text{m}$) at the stigma vs. flower petals, depending upon petal-opening angle. Thus, focused pollen deposition preferentially onto the stigma was theoretically confirmed. However, the finite time required to physically transport a charged pollen cloud into a flower (*i.e.*, ms time scale) is typically a million-fold longer than the ns time scale found to characterize floral charge relaxation. Insights achieved by this theoretical study thus redirected any further engineering R&D resources away from electrodynamically-focused deposition as based upon differential charge relaxation. Further work instead considered all flower components to be essentially grounded (*i.e.*, $\tau \sim 0$ s) and investigated the more conventional method for electrostatic deposition of charged pollen grains — especially onto geometrically protruding flower components such as the pistil where electric-field lines concentrate to provide increased deposition as compared to underlying surfaces.

While the preceding results were based upon electric fields created solely by the presence of a charged pollen cloud (*i.e.*, space-charge fields) and would be applicable to flowers located at all interior and exterior plant-canopy regions, other simulation studies modeled charged pollen trajectories towards an idealized flower under the influence of a field externally applied by a nearby high-voltage disk electrode (*i.e.*, a 2-D surface charge of $33 \mu\text{C}/\text{m}^2$ at a distance of 25 mm from a grounded flower simulating the approaching cloud of charged pollen). Computational results showed the applied electric field at the pistil tip to concentrate by 3-fold as compared with the field at the underlying flower structure, attaining a value of $160 \text{ kV}/\text{m}$ at the stigma. Considering natural inflorescence characteristics, these results confirm externally applied electric fields to be primarily of value for electrodeposition of charged pollen upon directly exposed stigmata.

Additional floral biophysics experiments showed no statistically significant detrimental effects upon pollen viability caused by exposure to electric fields exceeding 2 MV/m — an intensity 500-fold greater than the average field imposed on a pollen grain by a nearby charged honeybee. Furthermore, no detrimental electric discharges were observed to be induced from the tips or edges of flower components. Thus, electric-force management of grains in both natural and mechanized pollination processes should deliver viable pollen onto stigmatic surfaces with enhanced deposition efficiency.

Numerous binational research efforts were also directed for achieving the *second sub-objective* . . . *“to appropriately incorporate aerodynamic and electrostatic forces in the engineering design and development of a system for the mechanized harvesting and subsequent application of pollen on a commercial crop-production scale”*. Project investigators devoted considerable additional efforts in devising valid methodology to biologically assess the resulting electrostatic pollination processes and prototype machines under laboratory and field-test conditions. Following is an evaluation of research achievements in relation to meeting this second originally proposed sub-objective.

Basic to any electrostatic pollination process is the development of reliable means for accurately metering and electrically charging the viable pollen grains. This was achieved for pollen dispensed in both a dry-powder phase and in a liquid-carrier phase utilizing corona, triboelectric, and electrostatic-induction particulate-charging methods. Dry pollen grains dispersed in diluent powders were satisfactorily metered to charging nozzles by a fluidized bed/venturi injector apparatus. Extensive evaluations of possible biologically compatible dry diluents identified a castor-oil-derived polyamide 11 Nylon powder as ideal and several additional powders as suitable; no significant ($\alpha = 0.05$) reductions in germination occurred following 1-hour intermixing with pollen, or germination assays done in liquid medium containing leachates from these diluent powders. In contrast, germination was found to be partially-to-totally degraded for pollen dispersed in calcium carbonate and diatomaceous earth diluents.

To exploit the convenience of liquid metering, a biologically compatible carrier liquid was developed to suspend pollen for up to 30 min without degrading viability. Proper solute composition of this polyethylene glycol-based carrier ensured the balanced transmembrane osmotic potentials necessary to preclude cell rupture. Pollen at up to 5 % wt. could be dispersed in the carrier liquid

using Triton X-100 surfactant (unfortunately, later discovered to itself severely degrade pollen germination on stigmata). For a typical 2.5 mg/L pollen loading in the carrier, metering precision was experimentally found to be $\sim \pm 8\%$ over time.

When supplied to a pneumatic-atomizing electrostatic-induction spray nozzle, a liquid-carried almond pollen suspension was very satisfactorily charged to greater than 10 mC/kg charge-to-mass requiring only 1 kV nozzle input voltage; a linear ($R^2 = 0.98$) pollen-charging performance of 11 $\mu\text{C/kg}\cdot\text{V}$ was attained on a routine basis. The project thus achieved pollen-spray charging 10-times greater than the ~ 1 mC/kg at which electrostatic control of charged particulates (*e.g.*, pollen grains) typically becomes evident. Regarding biological impact, liquid suspensions of pollen which were atomized by aerodynamic shear at up to 276 kPa and induction charged in 1.6 MV/m electric fields within the nozzle maintained at least 80% germinability vs. control pollen. Consistent with goals of the research proposal, this induction pollen-charging capability incorporated properly balanced aerodynamic forces for trajectory of highly charged pollen grains to inner canopy regions for space-charge deposition.

Both triboelectric and ionized-field (*i.e.*, corona) charging methods were also satisfactorily developed for dry pollen by adapting industrial electrostatic-coating equipment for operation in the 50-1,000 mg/s powder flow range. Triboelectrification of pecan pollen to 7.6 mC/kg was achieved *via* multiple collisions with a Teflon charging sleeve at relative humidity values up to 48%. No electronic power supplies were required for this tribocharging of pollen, but the very low powder flow rates used have the disadvantage of creating little space-charge electric field for particle deposition onto stigmata at shielded regions within the canopy. High-voltage corona charging at 80 kV applied voltage on the nozzle satisfactorily charged date and almond pollen dispersed in several types of diluent powder. Because measured values of corona-nozzle currents are comprised to a large extent of the air-ion component vs. particulate charge, pollen charge values are not readily available; however, it is reasonable to assume a Pauthenier saturation pollen charge level of ~ 1 mC/kg was routinely attained. Air-assisted trajectory of corona-charged pollen was incorporated to increase deposition beyond peripheral regions of the canopy when using 40-80 kV applied corona-electrode voltages.

An overall evaluation of pollen-charging results confirms that this project has satisfactorily developed three distinct methods for electrostatic application of pollen. Assuming, for example, a

1 kV/cm electric field (readily attainable by space charge or applied electrode voltages) active on pollen grains possessing charge levels provided by the project-developed methods, then pollen-deposition forces 100-, 77-, and 10-fold greater than gravity have been achieved respectively by the induction, triboelectric, and corona systems.

Experimental tests of electrostatic pollen-application systems, developed using the corona and induction charging methods, were conducted under tightly controlled conditions within the laboratory and small-scale 1995-96 field trials on date palms and almonds. Extensive almond orchard trials were then collaboratively conducted in California during the 1996 and 1997 seasons. Unfortunately, Georgia apple orchard trials in 1997, using induction and triboelectric applications, were terminated by severe freeze damage to flowers. Following is an evaluation of research achievements of these 1995-1997 trials of electrostatic pollination methods/prototypes as they relate to the original research proposal.

Both induction and corona application methods were documented to significantly increase viable pollen deposition onto real stigmata and artificial targets by electrostatic attraction. As compared with uncharged pollen applications, air-assisted induction sprays (1 kV nozzle voltage) deposited 5.6-times more grains ($\alpha = 0.01$) as averaged across various target orientations while providing ~ 6 m/s canopy-penetration air velocity. Microscopic examinations of stigmata of induction-sprayed apple blossoms showed typically 3- to 5-fold greater supplemental pollen deposition for charged vs. uncharged sprays when assessed using methodology which ignored self pollen. Corona application (80 kV nozzle voltage) achieved 3.9-times greater pollen deposition ($\alpha = 0.05$) onto model pistil tops vs. zero-voltage uncharged application; very beneficially the intense applied deposition field from the 80 kV electrode concentrated 60-times more grains onto the protruding pistil top as compared with the underlying surfaces — emphasizing the appropriateness of high-voltage corona systems especially for exposed, non-shielded, peripheral target flowers. The effectiveness was further corroborated on exposed clusters of dates in preliminary 1995 orchard trials where overall percent fruit set on counted flowers averaged 30% for corona charged vs. 14% for uncharged pollen applications, giving a 2.1-fold significantly ($\alpha = 0.01$) greater date yield. Average fruit-set for date flowers not artificially pollinated was only 0.1%. Experiments for fruit set on individual branches of almond, conducted in Israel (Nachshon 1995, Yavneal 1996), gave an average increase of 105% and 45% respectively for the corona-charged pollen treatment. This fruit set

increase was in addition to the open bee pollination. The lower significance in yield increase found for the whole tree (as opposed to individual branches) might be related to the tree being close to its maximum production potential under the current conditions. These above laboratory and small-scale orchard trials thus achieved the project goal of demonstrating dramatically increased pollen depositions onto stigmata contributing to significantly increased crop yield *via* electrostatic pollination. Validation of these very positive results subsequently *via* large-scale orchard trials has been less certain under highly variable field conditions as seen below.

Large-scale 1996 almond orchard trials of electrostatic pollination generally failed to show statistically significant increases in fruit set or yield for either the dry-powder corona or the induction-sprayed pollen applications. Encouraging exceptions were: a) induction-charged pollen sprays supplementally applied twice at 800 mg/tree significantly ($\alpha = 0.05$) increased initial almond fruit set by 1.12-times that provided by open bee pollination, and although there was a steadily increasing trend toward higher percentage fruit set with increasing pollen dosage, the several lower dosage rates could not be statistically declared to set more fruit than open bee pollinations (*Note* - There remains the likelihood that the induction-sprayed almond pollen in 1996 had been rendered nonviable by the Triton X-100 surfactant used for its dispersion in the carrier liquid.); and b) uncharged pollen + talcum diluent supplementally applied by the corona system (0 kV) twice at 3.5 grams of pollen per tree significantly ($\alpha = 0.05$) increased almond fruit set by 1.87-times that provided by open bee pollination. (*Note* - Due to the well known Faraday shielding effect of 3-dimensional targets as often encountered with industrial corona-charging powder-coating systems, these 1996 almond tests failed to penetrate charged pollen beyond the tree's envelope and instead attracted it primarily to the outermost flowers. Without the 80 kV externally applied electric field, the uncharged pollen particles permeated the entire tree canopy and therefore reached many more flowers for enhanced pollination. While firmly documenting its ability to electrostatically deposit charged pollen onto directly exposed flowers of individually presented branches of dates, sweet cherry, and almonds, project results have clearly demonstrated the deterrent effect that high-voltage corona electrodes have upon tree canopy penetration, and corrective measures are being pursued.) Other potential problem areas encountered by the 1996 almond orchard trials included large tree-to-tree variability making it difficult to detect treatment difference above bee pollination, environmental

and biological factors not related to pollination (*e.g.*, extensive rain and fungal diseases) which may have masked treatment effects, as well as the surfactant and Faraday-shielding problems.

The 1997 almond orchard trials were redesigned to address certain of these problem areas. Electrostatic-induction charged pollen sprays without surfactant were applied to flowers in which bees were excluded by Delnet mesh bags, and early fruit set was evaluated shortly after the fruit abscission period associated with pollination status of the flowers. All treatments were applied to receptive flowers on a number of designated branches within the same tree and replicated on ten additional trees. A single application of induction-charged pollen spray produced 5% fruit set on receptive blossoms *vs.* a 23% value for open bee pollination (0% fruit set was measured for flowers which excluded both bees and spray). Thus, the 1997 trials verified that induction-charged pollen sprays can set fruit under field conditions to approximately 21% that achieved by bees; further efforts should increase the electrostatic pollination value by optimizing flower developmental stage for spray application, pollen dosage and number of spray applications.

V. DESCRIPTION OF COOPERATION

Overall, this project has been especially effective in drawing together US and Israeli researchers on both a professional and an interpersonal basis. It has also exemplified the creative benefits of interdisciplinary research collaboration across the botany-engineering interface. Thus, a high level of mutual respect and commitment to binational cooperation characterizes this BARD project as it concludes. All investigators would consequently welcome the opportunity for future collaborative research. A more detailed description of project cooperation follows in chronological sequence:

- A collaborative 2-week visit July 1993 by Dr. Gan-Mor of the Volcani Center with Prof. Law at the University of Georgia facilitated joint evaluations of electrostatics instrumentation needs and methods which subsequently allowed comparative measurements between work conducted in Israel and the U.S.
- A collaborative 4-week visit by Prof. Eisikowitch of Tel Aviv University with Professors Wetzstein and Law at the University of Georgia was arranged for the month of August 1994.

A concentrated effort was undertaken to experimentally determine what effects upon pollen viability are caused by exposure to intense electric fields. Much exchange of scientific information and learning from each other occurred throughout August with significant benefits to all involved parties.

- During September 1994 Prof. Law visited in Israel with Dr. Gan-Mor at the Volcani Center and with Prof. Eisikowitch at Tel Aviv University for consultations and review of progress regarding the collaborative BARD project. Pollen-charging technologies were jointly assessed, pollen-detachment prototypes demonstrated, and field evaluations tentatively planned.
- For the 10-month period January-August 1996, Prof. Eisikowitch was hosted as Visiting Researcher in the University of Georgia botany laboratory of Prof. Wetzstein with additional routine experimental investigations in Prof. Law's electrostatics laboratory. Numerous collaborative studies during this visit established proper methods, materials and protocols for dispersing viable pollen in osmotically/electrically appropriate spray-carrier liquids, and alternatively in biologically compatible dry-powder diluents, and documented the effects upon pollen deposition and germinability caused by the intense air-shear forces and electric fields imposed during the electrostatic application process. Concentrated studies during Prof. Eisikowitch's stay also cooperatively perfected the methodology for accurately assessing almond pollen germinability and grain count deposited onto flower stigmas as well as artificial target analogues.
- Under existing working relationships which Prof. Eisikowitch has with a major California grower of almond and pistachio nuts, collaborative field-testing programs for electrostatic pollination were jointly conducted on site by Israeli and US research teams during 12-15 day periods in February 1996 and 1997. Co-investigators Gan-Mor, Eisikowitch, Wetzstein and Law closely cooperated in the planning, preparation and execution of these orchard pollen applications *via* induction-charged sprays and corona-charged powders. Additionally, Professors Wetzstein and Eisikowitch cooperatively conducted an associated study of factors influencing almond fruitlet-dropping while on site.

- A joint US-Israeli authored research presentation of this BARD project was made before the international meeting of the Institute of Electrical and Electronics Engineers (Electrostatic Processes program session) in San Diego, California, October 1996. Two additional US-Israeli conference papers were scheduled as companion contributions for presentation September 1997 at the 5th International Symposium on Fruit, Nut, and Vegetable Production Engineering but, unfortunately, had to be withdrawn due to health and other crises within families of the lead authors.

VI. CONCLUSIONS

Project objectives have been achieved which will provide to agriculture the basic knowledge and the electrostatics technology needed for effectively applying viable pollen to flowering plants in a supplemental or alternative manner whenever traditional pollinating agents prove inadequate. Research results help address concerns regarding reductions in the populations and effectiveness of honeybee and other insect pollinators endangered by natural and man-made factors — *e.g.*, tracheal mite infestations, pesticidal decimation, Africanized bee intrusions, and unfavorable weather conditions — as well as increasing biological production efficiency in pollination-limited crops.

Detailed results and an evaluation of these research achievements have been presented earlier in Section IV summarizing the accomplishment of originally proposed objectives. Fundamental biophysical knowledge has been gained for understanding natural accumulations and transfers of electric charge and charged pollen during interactions of bees and flower components; this knowledge now provides a scientific basis for creating electrostatics-based mechanized pollination systems.

Three distinct processes have successfully been developed for electrically charging pollen grains in dry and liquid-carried states and electrostatically attracting the charged grains to grounded flowers for approximately 2- to 6-fold enhanced deposition onto stigmas. While this represents a significantly improved deposition efficiency, there remains much pollen wastage on non-stigmatic surfaces due to the inherent broadcast nature of supplemental mass pollination techniques. Insect-vectored pollen delivered to targeted flowers in anticipation of a nectar reward sets an exceedingly high standard for efficient pollen usage which is difficult for mechanized systems to attain.

Orchard trials have documented that electrostatically applied pollen sprays and powders can set fruit under field conditions with increases up to ~ 2 -fold. Additional work is required to ensure consistent increases in fruit set and corresponding crop yield, and to improve whole-tree penetration of electrostatically-applied pollen. Much opportunity also remains for investigating the biochemical interactions/signalings associated with diluent powders and carrier liquids deposited onto stigmatic exudate and the biological implications regarding pollen germination, pollen-tube growth, and fertilization.

VII. LIST OF PUBLICATIONS

BARD collaboration has led to the following journal articles, refereed proceedings and conference papers relating to electrostatic processes underlying natural and mechanized transfer of pollen.

- Gan-Mor, Samuel and S. Edward Law. 1992. Frequency and phase-lag effects on transport of particulates by an AC electric field. *IEEE Trans. IA-28*(2):317-323.
- Law, S. Edward. 1993. Transient characteristics and electric boundary effects controlling electrostatic crop spraying. *Proceedings of 6th Annual Conf. of Inst. for Liquid Atomization and Spray Systems*, pp. 55-59. Worcester, MA.
- Gan-Mor, S., Y. Schwartz, A. Bechar, D. Eisikowitch and G. Manor. 1995. Relevance of electrostatic forces in natural and artificial pollination. *Canadian Agricultural Engineering* 37(3):189-194.
- Dai, Y. and S. Edward Law. 1995. Modeling the transient electric field produced by a charged pollen cloud entering a flower. *IEEE/LAS Conf. Record 2*:1395-1402. ISBN 0-7803-3008-0.
- Banerjee, S. and S. Edward Law. 1995. Triboelectric charging of bioparticulates. *Proceedings of the 1995 Conference of the Electrostatics Soc. of America*, pp. 55-56. Rochester, NY. ISBN 1-885540-01-9.
- Banerjee, S. and S. Edward Law. 1995. Characterization of chargeability of biological particulates by triboelectrification. *IEEE/LAS Conf. Record 2*:1295-1301. ISBN 0-7803-3008-0.
- Law, S. Edward, S. Banerjee, Hazel Y. Wetzstein and D. Eisikowitch. 1996. Electrostatic application of pollen sprays: effects of charging field intensity and aerodynamic shear upon deposition and germinability. *IEEE/LAS Conf. Record 4*:1940-1948. ISBN 0-7803-3544-9.

- Bechar, A., I. Shulevich, D. Eisikowitch, Y. Vaknin, B. Ronen and S. Gan-Mor. 1996. Modeling and experimental analysis of electrostatic date pollination. *ASAE Conf. Paper No. 96-0184* (microfiche), St. Joseph, MI.
- Banerjee, S. and S. Edward Law. 1996. Electrostatic induction charging of pollen suspensions. *IEEE/LAS Conf. Record 3:1775-1781*. ISBN 0-7803-3544-9.
- Law, S. Edward, J. Robert Cooke and Steven C. Cooper. 1997. Space charge suppression of electrostatic-induction spray charging. *Jour. of Electrostatics* 40&41:603-608.
- Wetzstein, Hazel Y. and S. Edward Law. 1997. Enhanced pollination: critical considerations at the biological-electrostatics interface. *Proceedings of the 25th Annual Conference of the Electrostatics Soc. of America*, pp. 14-15. Athens, GA. ISBN No. 1-885540-05-1.
- Cooke, J. Robert and S. Edward law. 1997. Finite element analysis of space charge suppression of electrostatic-induction spray charging. *IEEE/LAS Conf. Record 3:1756-1763*. ISBN No. 0-7803-4067-1.
- Cooper, Steven C. and S. Edward Law. 1997. Commercialization of liquid electrostatic spraying systems for agricultural applications. *Proceedings of the 25th Annual Conference of the Electrostatics Soc. of America*, pp. 30-31. Athens, GA ISBN No. 1-885540-05-1.
- Law, S. Edward, Hazel Y. Wetzstein and Dan Eisikowitch. 1997. Electrostatically enhanced pollination process for nut and fruit trees. *Proceedings of 5th Internal. Symposium on Fruit, Nut, and Vegetable Production Engineering*. Davis, CA (withdrawn for medical reasons).
- Law, S. Edward and Hazel Y. Wetzstein. 1998. Pneumatic atomization and induction spray charging of aqueous based viable pollen suspensions. *Proceedings of 11th Annual Conf. of Inst. for Liquid Atomization and Spray Systems* (in press). Sacramento, CA.

VIII. REPORT ON PATENTS

Several patent applications have been filed by Israeli and US investigators relating to this BARD project's requirements for accurate metering of dry-powder-carried pollen and for improved induction-charging of liquid-suspensions of pollen.

- Law, S. Edward and Steven C. Cooper. 1998. Electrostatic induction spray-charging nozzle system. U.S. Patent No. (pending). U.S. Patent Office, Washington, D.C.
- Cooper, Steven C. and S. Edward Law. 1998. Electrostatic spray nozzles for abrasive and conductive liquids in harsh environments. U.S. Patent No. 5,704,554. U.S. Patent Office, Washington D.C.