J. Amer. Soc. Hort. Sci. 104(2):240–243. 1979.

A Morphological Study of the Development of Reproductive Structures of *Phaseolus lunatus* L.[1]

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Additional index words. lima bean, pollination, floral morphology, scanning electron microscope

Abstract. **Lima bean, *Phaseolus lunatus* cv. Early Thorogreen, is primarily self-pollinated. Morphological characteristics of reproductive structures which facilitate self-pollination include the stage of floral development at the time of anther dehiscence and the relative positions of anthers and stigma within the keel at the time of pollen shedding. Coincidental maturation of pollen and receptivity of the stigmatic surface also enhance the capacity for self-pollination. The varying degrees of cross-pollination frequently reported may be facilitated by extrusion of the stigma from the keel, which occurs in connection with insect visitation at anthesis, and also by the prolonged period of stigma receptivity, which extends from the white bud stage through anthesis.**

This paper is concerned with the morphogenesis of reproductive structures of *Phaseolus lunatus*, the lima bean, and the relationship between the development and maturation of its reproductive organs and its mode of pollination.

Interest in the reproductive biology of *P. lunatus* arises primarily from the concern of plant breeders to broaden the genetic base of bean cultivars and to enhance the potential for resistance to bacterial, fungal and virus pathogens (10). To some extent the interest issues also from the extraordinary difficulty encountered in obtaining successful manual cross-pollinations. *Phaseolus lunatus*, like *P. vulgaris*, is regarded as a self-pollinated species (5,7,12,21). Development of cross-pollinating populations, which has been a concern of Ibrahim and Coyne (10), Zaumeyer (25) and others is considered to be potentially useful in connection with plant breeding programs designed to increase gene recombination and to develop new favorable linkages.

The self-pollinating characteristic of *P. lunatus* has been variously related to the locale of the crop and the climatic conditions prevailing during the growing season; to the proximity of potentially compatible species; to the prevalence and variety of insects visiting the plants; to the quality and quantity of pollen produced; and to the time of anther dehiscence and anthesis (2,8,15,16). The dearth of definitive information about pollination and reproductive development in *P. lunatus* and the potential usefulness of cross-pollinating populations prompted this investigation.

Materials and Methods

Buds and flowers from the cultivar 'Early Thorogreen' lima beans were collected during the 1977 and 1978 growing seasons from plants in the field at Davis, California, growing under standard cultural conditions, and from plants grown in the growth chamber at 27 ± 2°C with 21.6 klx of light for a 16 hr photoperiod. Fresh samples were dissected under a microscope to determine the form and orientation of the androecium and pistil in relation to that of the standard, wing and keel petals at various stages of bud and flower development. Petals were removed with extreme care in order to retain the normal positions of the male and female reproductive structures during bud development and at anthesis. Significant morphogenetic changes in the development of the androecium and pistil were recorded using a Zeiss Tessovar photomicroscope.

Techniques for scanning electron microscope study were modified from those developed by Webster et al. (21) and Lynch and Webster (14). Those involved fixation of dissected buds and flowers in 50% formalin-propionic acid-alcohol (FPA), subsequent dehydration through an alcohol series to 100% ethanol, followed by replacement with amyl acetate for 1 hr in preparation for critical point drying with carbon dioxide. After 2 hr of drying samples were mounted on stubs using polyvinal chloride adhesive (13) and shadowed with gold before viewing. Microscopic observations and photographs of reproductive structures from buds and from flowers at anthesis were made using a Cambridge Stereoscan Electron Microscope.

Results

Floral characteristics. The hermaphroditic, papilionaceous flowers of a determinate type lima bean, such as 'Early Thorogreen', are borne on axillary and terminal few-flowered racemes. Bracts and calyx are small; the latter is campanulate with very small teeth. The corolla is also relatively small, and in 'Early Thorogreen' the standard, wing and keel petals are white. The keel, the distinctive morphological characteristic of the genus, is prolonged in *P. lunatus* into an elaborate spiral. This encloses the androecium and pistil.

The androecium is comprised of 10 stamens. Nine of these are connate and the tenth (the vexillary stamen) is free. The anthers are relatively small; during development and at maturity they appear uniform in size. Pollen is dehisced simultaneously by all anthers. The filament is linear and its apical region is dilated to a small extent.

The stigma of the pistil is slightly subterminal and adaxially situated. The stigmatic pad is covered by epidermal cells, some of which are papillate and others of which are modified as hairs. The apical region of the style is linear and it is pubescent both adaxially and abaxially. A brush of hairs extends along most of the adaxial length and along part of the abaxial portion of the style. The ovary is elongate, superior, and densely pubescent. Hairs along the style and on the ovary are oriented so that tips point toward the stigma.

Buds open early in the morning and through part of day. The flower does not close, but the corolla is shed after 2-3 days. Nectar is secreted by a discoid nectary at the corolla base. Bees visit the flowers for both pollen and nectar.

Our preliminary observations indicate that the morphological development and the spatial arrangement of the androecium and pistil are different in green buds, small and large white buds, and open flower stages of floral development.

Green buds. Both stamens and pistil of green buds are immature. The pistil is distinctively differentiated into a stigma, style, and ovary (Fig. 1). The stigma appears as a small, flat-

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[1] Received for publication August 29, 1978. Research supported in part by funds from the California Dry Bean Advisory Board. The authors acknowledge with gratitude the assistance of Susan Schmickle and Nancy Dehgan.

The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper must therefore be hereby marked *advertisement* solely to indicate this fact.

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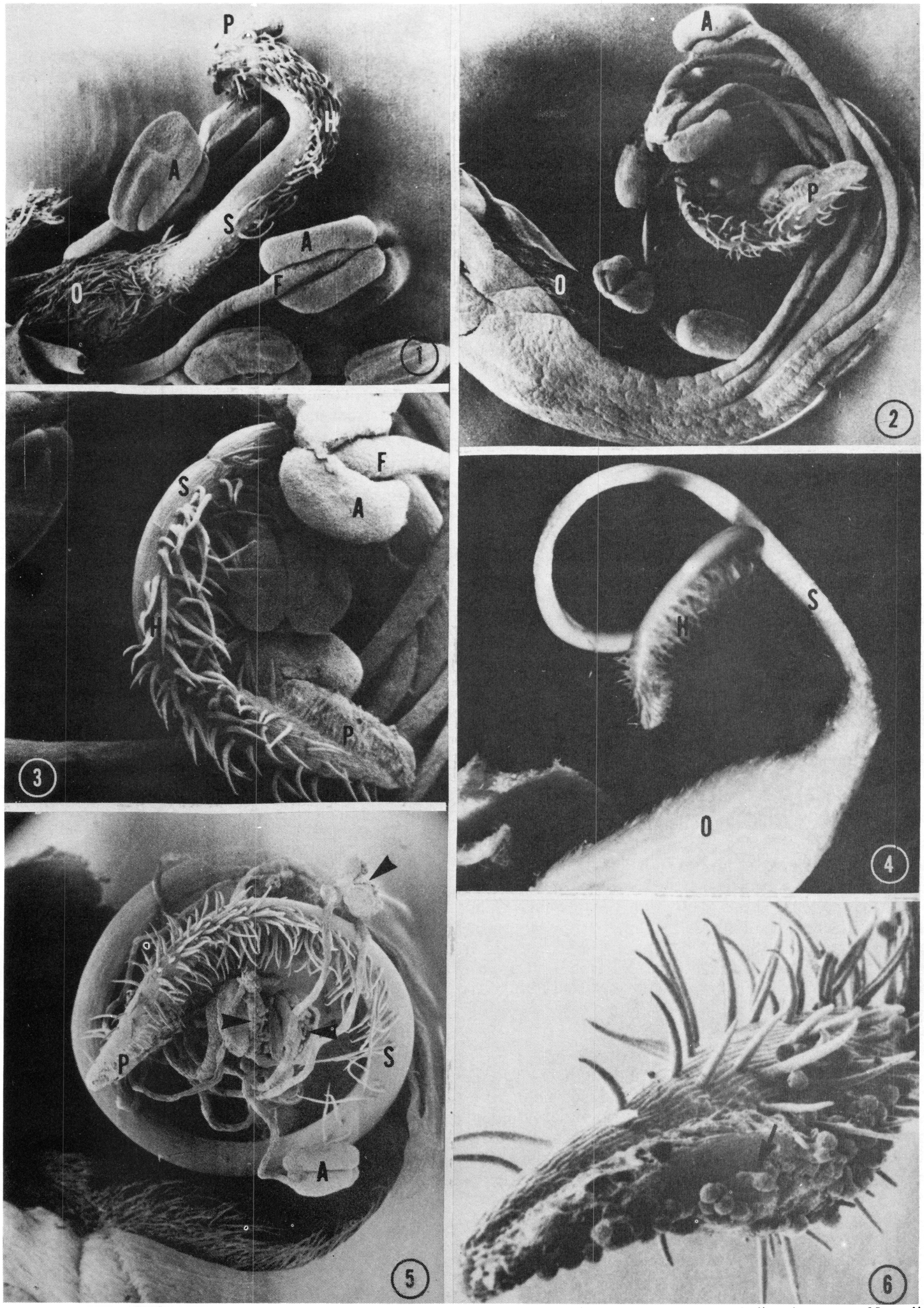

Figs. 1-6. Developmental stages of the reproductive structures of *P. lunatus.* Fig. 1. Green bud stage. Immature pistil and stamens. Note difference in length of pistil and stamens and hairs on the style and ovary oriented toward the stigma. Anthers are twisted so that the region of pollen dehiscence faces the style and stigma. X60. Fig. 2. Early white bud stage. Maturing pistil and stamens prior to anther dehiscence, showing connate stamens with elongated, coiled filaments. Note stigmatic pad at the tip of the elongated, coiled style, oriented so that it faces the upper part of the keel, and positional relationships of the anthers and the stigma. X25. Fig. 3. White bud stage. "Wet" stigma, positioned adaxially and surrounded by stigmatic and stylar hairs, and anthers oriented so that regions of dehiscence are proximal to the style. X60. Fig. 4. Tessovar photograph of the pistil in the large white bud stage, showing the double coil of the style and the receptive surface of the stigma facing the base of the keel. X30. Fig. 5. Large white bud stage. Note dehiscence of anthers and shedding of pollen (arrows). Note also positions of the stigma and anthers at the time of pollen dehiscence and the collapse of stamen filaments as pollen is shed. X35. Fig. 6. Open flower stage. The mature, receptive stigma, with pollen grains adhering to it and to adjacent stigmatic and stylar hairs. Arrow indicates germinating pollen grain. X90. A = anther; F = filament; H = hairs; O = ovary; P = stigmatic pad; S = style.

tened adaxial pad, which is conspicuous by its tiny, protruding epidermal cells and the fact that it is glabrous. It is delineated by a margin of short, rigid hairs. The style is linear and curved approximately one-third of its length below the stigma. It is slightly twisted, and most of its adaxial surface is covered by a dense mat of hairs (Fig. 1).

Stamens are differentiated into anthers, containing immature pollen, and filaments. The latter are twisted and bent so that regions of dehisence of the anthers face toward the pistil (Fig. 1). Since the total length of the pistil exceeds that of individual stamens, the anthers are positioned proximal to the style. Both the stigma and upper stylar region protrude beyond the stamens.

White buds. The stigma of the pistil of the small white bud is regarded as mature. Its surface is covered with material of a mucilaginous consistency which is secreted by the subtending epidermal and subepidermal cells (Fig. 2,3). Stigmatic epidermal cells thus comprise an essentially glandular tissue. According to Esau (6) such a "wet" stigma resembles a nectary in structure and function.

The style is elongate, coiled and twisted. As a result of these morphological modifications the stigmatic surface is oriented toward the upper part of the keel. The stigma and distal stylar regions project into a different plane from that of the stamens (Fig. 2). Stylar hairs are conspicuous, distributed adaxially and abaxially in the region of the stigma and adaxially along the remaining length of the style (Figs. 3,4). Extensive filament elongation and coiling result in anthers being positioned randomly above and below the stigma and the apical stylar region.

In the enlarged white bud, stylar elongation and coiling orient the stigma so that its receptive surface faces toward the base of the keel, or, more precisely, toward the juncture of the style and ovary (Fig. 4,5). At this stage anthers dehisce and pollen is shed (Fig. 5). Most of the pollen lodges in the stylar hairs or falls to the sutending portion of the keel. Some pollen grains do adhere to the stigmatic surface; others are held among the hairs adjacent to the stigmatic surface (Fig. 6).

Fully open flowers. The stigmatic pad in the fully open flower is within the keel, oriented so that it faces the base of the coiled keel. The stigma and apical portion of the style can be forced to protrude through the keel of the open flower by depression of the wing petals. This occurs in the field when insects visit the flowers. The extruded stigma is still viable, i.e., it is receptive to manually applied pollen of *P. lunatus,* which germinates on the stigmatic surface (Fig. 6). This is an important factor in connection with the possibility of successful outcrossing by insect visitors.

Discussion

Phaseolus lunatus is categorized as a self-pollinated species (5,7,12,18,20). This study suggests that the self-pollination mechanism is attributable to: 1) the stage of floral development at the time of pollination; 2) the relative positions of the anthers and stigma within the keel at the time of pollen dehiscence; and 3) the coincidental maturation of pollen and stigma.

However, the literature on *P. lunatus* is replete with reports of outcrossing to varying degrees. According to Harding and Tucker (8), the frequency of outcrossing ranges from less than 1% to greater than 10%. Occasional high rates of outcrossing (to 89%, e.g., depending on the variety and growing conditions), command attention primarily because of the possibility of significant yield increase, hybrid vigor, the generation of genetic variability, and contamination of pure seed lots (3,5,16,22,23, 24). Lambeth (12) noted that mechanical tripping to ensure adequate pollination in *P. lunatus* is unnecessary, but Free (7) stated that in his estimation no adequately controlled experiments have yet been carried out to investigate the effect of insect pollination in increasing yield.

We regard our studies on the propensity of cross-pollination in *P. lunatus* as preliminary, but there are two observations we deem of particular importance: 1) cross-pollination appears to be mechanically facilitated by extrusion of the stigma at anthesis in connection with insect visitation; and 2) the extruded stigma is receptive to pollen of *P. lunatus* (pollen grains adhere to the stigmatic surface and will germinate there).

The extended period of stigma receptivity of *P. lunatus* is interesting not only in relation to the potential for outcrossing but because the interval of receptivity differs markedly from that of the *P. vulgaris* stigma. The latter is also regarded as a self-pollinated species and interspecific crosses between *P. vulgaris* and *P. lunatus* have been attempted with varying degrees of success (1,9,20). Our observations indicate that at anthesis the stigma of *P. vulgaris* is partly dried and receptivity to pollen appears negligible (21). Although remnants of germinated pollen grains are occasionally visible on some portions of the *P. vulgaris* stigma at anthesis, there is no evidence of fresh pollen on either the stigma or stylar hairs (21). This contrasts markedly with the appearance of the stigma of *P. lunatus* at anthesis. These observations, as well as those on the morphogenesis of reproductive structures in both species bear upon Tucker and Harding's (19) report of near zero outcrossing in California-grown populations of *P. vulgaris* and on the reports of varying degrees of outcrossing in *P. lunatus* (8,22,23,24). Our observations also relate directly to the potential for outcrossing—a potential which would appear to be enhanced in *P. lunatus* as a result of stigma receptivity at anthesis.

The cross-pollination process in *P. lunatus,* as described by Magruder and Wester (16), involves a "slit in the membrane," which we interpret to mean an opening in the keel. To reach the slit an insect alights on the keel or wings, climbs up over them, and in so doing depresses them. This forces the stigmatic surface and sometimes one or more of the stamens out the end of the "enveloping membraneous sheath."

Our observations do not agree with this description. We have observed stigma protrusion at anthesis in connection with insect visitation but not extruded stamens. In fact, we have noticed that after pollen dehiscence in the large white bud stage in *P. lunatus,* anthers and filaments shrivel and stamens collapse. This situation accords more nearly with the view of Knuth (11) who has stated that the stigma and a portion of the style with pollen adhering to the brush of stylar hairs project when the keel is depressed and retract when pressure is removed. Thus it appears that the potential for outcrossing, from a mechanical and morphogenetic point of view, is related primarily to the pistil.

Finally, one extremely interesting aspect of a study of the reproductive biology and pollination mechanisms of *P. lunatus* is the pollinators. A number of different kinds of bees, both social and solitary, visit populations in the field at Davis, and bees have been credited as effective pollinators (2,16,17). Allard (2), Bailey (4), and Mackie and Smith (15), though, have concluded that thrips (*Frankliniella occidentalis*) are involved in outcrossing. Considering the relative size of thrips and of pollen grains, and the flight pattern of thrips, their role as effective pollinators should be investigated further.

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J. Amer. Soc. Hort. Sci. 104(2):243–245. 1979.

Effect of Thrips Scars on Table Grape Quality[1]

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Additional index words. Vitis vinifera, Frankliniella occidentalis, grape pests, integrated pest management

Abstract. **Scars caused by the ovipositional and feeding activities of the western flower thrips, *Frankliniella occidentalis* (Pergande), on 'Thompson Seedless' and 'Calmeria' table grapes, *Vitis vinifera* L., had no effect on many measurable quality attributes of the fruit. Scarred berries showed no apparent differences in size or average weight when compared to undamaged fruit. However, scarred 'Thompson Seedless' berries had a higher soluble solids content. The acid content was not affected by any type of scar and all fruit had soluble solids to acid ratios of at least 20:1. Scarring did not affect the weight loss of fruit in short-term storage at 0.6°C, and scarred berries were not injured by sulfur dioxide fumigation.**

Of many arthropod pests found on grapes, the western flower thrips, *Frankliniella occidentalis* (Pergande), is reported to damage the foliage and fruit (3, 10, 11). Damage to the fruit results from the feeding or ovipositional activities of the insect (4). Scars on the surface of the fruit are believed to be caused by thrips that feed under floral caps which adhere to the berries (6, 14). However, scars may also be caused by gibberellin sprays or abrasion (7, 14).

Scars are visual imperfections that detract from the appearance of the cluster. The damage is unimportant in grapes used for raisins and wines, but scars on table grapes can be objectionable. When the injury is extensive the fruit is not marketable because of high consumer expectancies and legal standards of quality that restrict insect damage (2).

Insecticide control practices are often directed at phytophagous insects that affect the appearance of the fruit (1). In the concept of integrated pest management a certain level of insect damage must be tolerated in order to decrease the use of insecticides. Superficial damage also referred to as cosmetic damage caused by insects in fresh produce may not actually affect the food value of the commodity. This possibility has not been investigated and such information is needed to develop tolerable insect damage levels. Although thrips damage on table grapes is only an occasional problem, this study is an attempt to determine the effect of scars on storage properties and other objective attributes of table grape quality.

Materials and Methods

Table grapes. In 1972 samples of mature 'Thompson Seedless' table grape clusters were obtained from a commercial vineyard in Tulare County, California. Samples of 'Calmeria' table grapes were taken from a vineyard in Fresno County, California. The clusters were selected for fruit scarred by the feeding and ovipositional activities of thrips. The samples were held in plastic bags at about 0.6°C during the 6 week period in which they were evaluated.

Evaluation of scar damage. For quality evaluation studies individual berries were removed from the cluster by cutting the pedicel about 5 mm above the top of the fruit. Berries with

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[1] Received for publication June 27, 1978. Research supported in part by grant in aid funds to Dr. R. L. Doutt, Professor Emeritus, Division of Biological Control, University of California, Berkeley, from the California Table Grape Commission.

The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper must therefore be hereby marked *advertisement* solely to indicate this fact.

[2] Present address: Department of Biology, California State University, Long Beach, CA 90840. The author is grateful to the late Dr. M. Uota, USDA, ARS, Horticultural Crops Production Research Station, Fresno, CA, for providing his assistance and research facilities to accomplish this project, and to Dr. R. L. Doutt, Professor Emeritus, Division of Biological Control, University of California, Berkeley, for his support and sponsorship, and to Mr. F. L. Jensen, University of California San Joaquin Valley Agricultural Research Extension Center, Parlier, CA, for providing the 'Thompson Seedless' grapes.