Winter 1993

Characterization and inheritance of the fused vein trait in squash (Cucurbita pepo L)

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Order Number 9420561

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University of New Hampshire, 1993

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CHARACTERIZATION AND INHERITANCE OF THE FUSED VEIN TRAIT IN SQUASH (*CUCURBITA PEPO* L.)

by

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B.S., Dickinson College 1979

DISSERTATION

Submitted to the University of New Hampshire
in Partial Fulfillment of
the Requirements for the Degree of

Doctor of Philosophy

in

Genetics

December, 1993

This dissertation has been examined and approved.

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10/25/93
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DEDICATION

This dissertation is dedicated to Jim Matheson and Bryant Long who introduced me to the art of plant breeding, trained my horticultural eye, trusted my abilities and judgement, and encouraged me to continue my education.

ACKNOWLEDGEMENTS

This research was made possible by the encouragement, advice, and support of many family, friends and colleagues. I am especially indebted and sincerely grateful to Dr. J. Brent Loy, for serving as advisor and committee chairman. His friendship, and enthusiasm for plant breeding will always be valued and remembered.

Appreciation is also extended to Dr. Yun Tzu Kiang, Dr. Tom Lee, Dr. Tom Davis, and Dr. Jim Pollard, for reviewing and evaluating this research and dissertation. Their combined wisdom and guidance will serve me for years to come.

I am also grateful to John McLean, Bob Parker, Suzy Wyrostek, Matt Newton, John Breen and the Horticulture Farm Crew for the attention and care given to my plants. Despite pests, plagues, storms and droughts their efforts kept my research alive.

I also wish to thank the Department of Plant Biology, the Genetics Program, the College of Life Sciences and Agriculture, and the Graduate School of the University of New Hampshire for providing financial assistance, facilities and the opportunity to conduct this research.

Lastly, special thanks are extended to Thelma Stolzenburg who served as sounding board and confidant and to Emily and Charles Hart who provided great friendship, essential diversion and a garden sanctuary.

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ABSTRACT

CHARACTERIZATION AND INHERITANCE OF THE FUSED VEIN TRAIT IN SQUASH (CUCURBITA PEPO L.)

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This investigation characterized and determined the inheritance of the fused vein trait in *Cucurbita pepo* L. with the purpose of evaluating its use as a rouging marker for hull-less seeded cultivars.

The trait is characterized by the partial fusion of the primary leaf veins. There is a reduction in the intraveinal leaf blade and the dorsal leaf surface appears wrinkled. Depending on genetic background, fusion begins with the fourth to tenth leaf stage and continues throughout vegetative growth. The extent of fusion also varies from slight (1-5 cm) to pronounced (15-20 cm). Anatomically, fusion results from an interruption of vein formation. In the transition zone between petiole and leaf blade, the normal pattern of vascular coalescence and dispersal is expanded into two cycles.

Analysis of segregating populations demonstrated that the trait is most likely governed by a single recessive gene, *fv*, linked to the hull-less seed gene, *n*, with 29.2 map units between them. However, inheritance ratios were distorted, varying with population, environment, and type of cross. A hypothesis of gametophytic subvitality was verified by microscopic examination of co-pollinated, style-partitioned flowers, revealing

inferior fused vein gametophyte performance.

The consequences of gametophytic subvitality were shown by manipulating reproductive competition. Fused vein pollen generated significantly fewer seed per fruit in all female genotypes when compared to normal, F_1, and a 50:50 pollen mix at three different pollen loads. In ensuing F_2 and testcross populations, a reduction in pollen load and therefore pollen competition significantly increased the number of fused vein individuals in segregating populations. Plant growth was also assessed for negative pleiotropic effects. No significant difference was found between fused vein and normal individuals in segregating populations; both leaf initiation and total leaf area were independent of leaf morphology.

The trait's distinct morphology, recessive inheritance, and linkage relationship make it a suitable rouging marker. However, gametophytic subvitality prescribes fused by normal hybrid cultivars to avoid detrimental effects on yield.

CHAPTER I

INTRODUCTION

"Thou shalt not sow thy fields with mingled seed" (Leviticus 19:19).

The success of modern agriculture can be attributed, in part, to the development and use of superior uniform cultivars. Uniformity of maturity, plant habit, and economic characters enhances agricultural mechanization, standardizes production and reduces labor requirements. The genetic purity of a commercial seed lot is important to maximize uniformity and realize the advantages of modern agriculture. Although Federal and State truth-in-labeling laws set minimum standards for seed quality and purity, the demands of growers, processors and consumers establish higher premiums for seed purity (Schiffman and Schery, 1961; Rollin and Johnston 1961).

The seed industry spends considerable resources to maintain and insure cultivar purity (McCorkle and Reed, 1961). Their efforts are designed to reduce the effects of natural mutation and to avert contamination by outcrossing or physical mixing. The continual production of pure seed requires: trueline selection, stringent isolation, repeated roguing, careful harvesting and processing, and various purity screens and grow-outs.

The ability to maintain and test a cultivar's purity is enhanced by its uniqueness. Cultivars can be distinguished by biochemical constit-

uents, disease resistances, isozyme patterns, RFLP patterns, morphological characters and/or agricultural attributes. For economic reasons, the seed industry relies heavily upon morphological and agricultural traits to identify cultivars. Detection of contamination during large acreage seed production is based almost exclusively on gross changes in visible plant morphology.

Uncommon single gene recessive traits expressed prior to a crop's reproductive stage are particularly useful for detecting contamination. Uncommon traits provide detection from a relatively broad range of potential contaminants. Single gene recessives are easily manipulated in breeding programs and reveal contamination in the first generation after outcrossing. Expression prior to a crop's reproductive stage permits removal or rouging before further contamination of stocks can occur.

Few genes are known for *Cucurbita pepo* L. that can be used to detect outcrossing. Although *C. pepo* is among the oldest of domesticated crops and has been selected and bred extensively, only 36 genes have been characterized (Robinson and Hutton, 1992). Of these, two recessive forms have possible value as rouging markers: leafy tendril, *lt*, (Scarchuk, 1974), and rosette leaf, *ro*, (Mains, 1950). They are both rare recessive morphological markers expressed prior to flowering. Two markers, however, are insufficient to distinguish the hundreds of available *C. pepo* cultivars.

A new mutant leaf form, fused vein, has potential as an additional *C. pepo* marker. It was first noticed by Dr. J Brent Loy in 1987 among hull-less seeded breeding material descending from a cross between

"Trickyjack" and "Minijack" (Figure 1.1). The trait is readily distinguishable, is expressed prior to flowering, is genetically stable, and has no apparent effect on fruit yield.

The fused vein trait was a fortuitous find among hull-less seeded material. The hull-less seeded character is a maternally expressed recessive (Stuart, 1983). A cross between a hull-less female and a hulled male yields F_1 seeds that are phenotypically hull-less but genotypically heterozygous. The resulting F_1 plants, when self pollinated, produce phenotypically hulled F_2 seeds that are a mixture of homozygous hulled, heterozygous and homozygous hull-less genotypes. Although these F_2 hulled seeds can be distinguished and physically separated from hull-less seeds, an outcrossed F_1 plant in a production field spreads hidden contamination that is expressed in the following generation. Because many *C. pepo* cultivars have similar juvenile morphologies, production of hull-less seeded cultivars has been prone to repeated contamination (Matheson, 1991). By limiting the use of a rouging marker, like the fused vein trait, to hull-less seeded cultivars, contamination by hulled material can be minimized.

The objectives of this study were to: 1) characterize the morphology and anatomy of the fused vein trait, 2) determine its mode of inheritance, 3) evaluate its effect on gametophyte fecundity, and 4) assess its usefulness as a rouging marker.

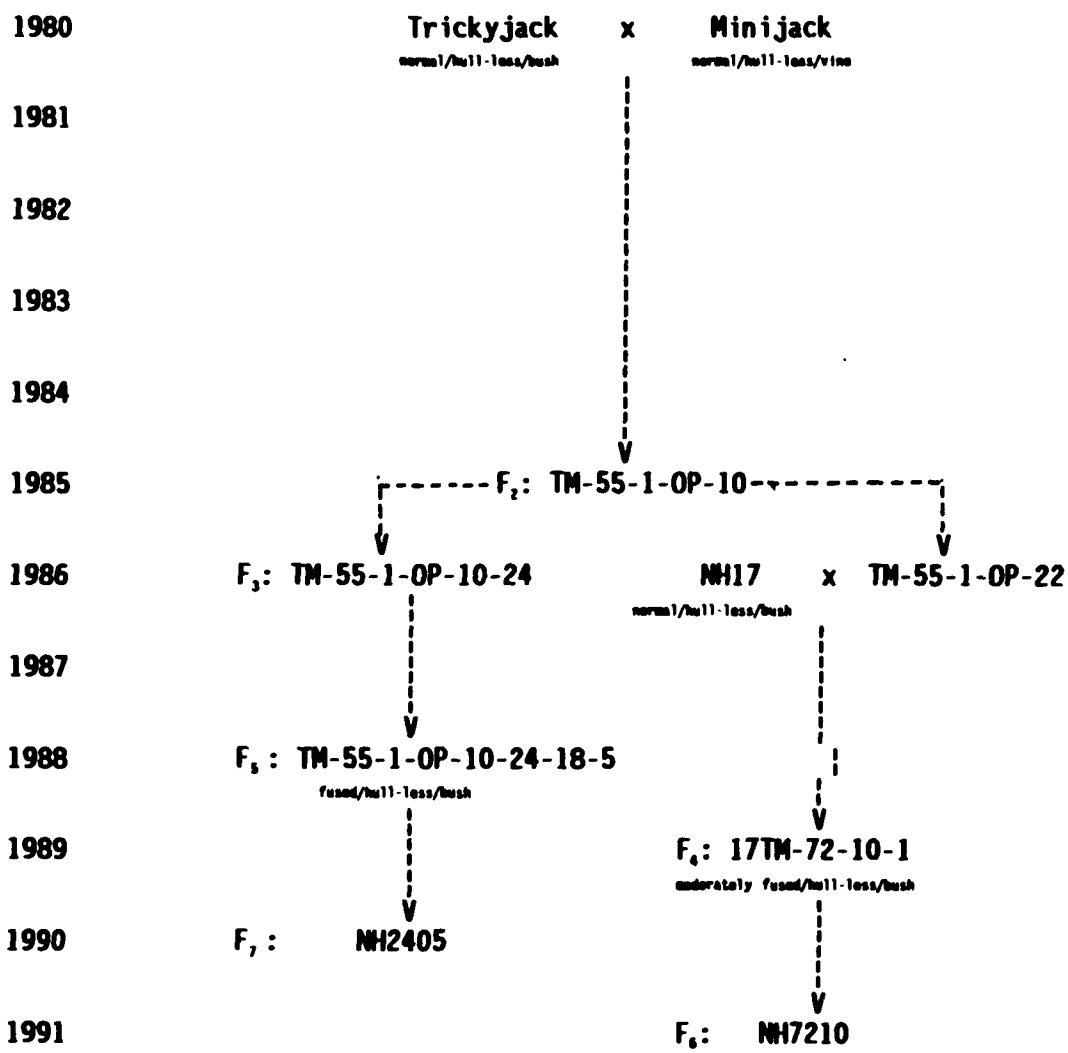


Figure 1.1. Pedigrees for the fused vein inbred NH2405 and the moderately fused vein inbred NH7210.

CHAPTER II

LITERATURE REVIEW

Part I: *Cucurbita pepo* L.

General Morphology

Cucurbita pepo L. is a large, rapidly growing, annual species that is frost sensitive but tolerant of cool temperatures. It exhibits considerable variation in both vegetative and reproductive structures. The principle morphological studies of *C. pepo* were conducted prior to 1940 and were reviewed and expanded upon by Whitaker and Davis (1962). Their description is complete and provides the basis for the following discussion.

C. pepo's plant habit ranges from a compact single stem bush to a rambling multi-stem vine. The stems are angled, semi-erect or trailing, and are covered in varying degree with harsh bristles or spines. The leaves are borne alternately and upright. The leaf blades are simple, palmately lobed, serrated, and large, often exceeding 24 cm in width. Juvenile leaves are frequently three-lobed while mature leaves have five shallow to deep lobes with narrow to wide sinuses. Associated with each lobe are predominate veins or midribs. The midribs are raised from the ventral surface of the blade forming ridges that taper distally. The petioles are spiny, hollow, and long, more than twice the length of the blade.

C. pepo is chasmogamous, moneocious and bee pollinated. Staminate and pistillate flowers are borne singly in the axils of the leaves. The flowers are large, showy, bright yellow to orange, and bloom for only one day. The perianth is composed of a calyx of five short narrow sepals and a campanulated corolla of five large lobes that alternate with the sepals. Staminate flowers are borne on slender pedicels and bloom sequentially from the center of the plant outward. The androecium is short thick and conical; it is composed of three stamens united by their anthers. Pistillate flowers are less numerous then staminate flowers and are borne on short thick peduncles. They are found distal to the staminate flowers but follow the same sequential pattern of bloom. Pistillate flowers are epigynous and consist of three cohesive carpels with a corresponding number of locules. The style is short, thick, and terminates in three bilobed papillate stigmas.

C. pepo fruit are indehiscent pepos; they consist of fleshy endo- and mesocarps surrounded by a hard rind. They vary greatly in size, shape, color, and texture, which is illustrated by the nine recognized cultivar groups: pumpkins, scallops, acorns, crooknecks, straightnecks, marrows, cocozelles, zucchinis and ornamental gourds (Paris, 1989). Fruit can be white, green, yellow or orange; striped or solid; warty or smooth; hard or soft shelled; spherical, cylindrical, or disk shaped. The flesh can be moist or dry, fine or coarse fibered, and white, cream, or yellow. Although varying in length, *C. pepo* 's mature peduncle is a distinguishing taxonomic feature within its genus; it is hard, sharply five angled and without cork. *C. pepo* seeds are white to tan, ovate-ellipsoid and

separated easily from the fruit pulp. They vary in size from seven to twenty millimeters in length. Seeds of some cultivars lack well developed seed coats and their embryos are enclosed in dark green inner seed coat.

Leaf Anatomy

Metcalfe and Chalk (1950) and Whitaker and Davis (1962) assembled anatomical descriptions of *C. pepo* leaf structure from studies prior to 1940. Their descriptions lack specific details but are still current. Subsequent investigations have been sporadic and limited to the classification and comparison of stomata and trichomes in the Cucurbitaceae.

The mature *C. pepo* leaf blade exhibits subtle variations in structure. Although the epidermal layer is primarily one cell thick, it becomes two to three cells thick in ventral areas near the midribs. The palisade mesophyll comprises sixty percent of the blade's thickness but is arranged in both one and two cell layers. The spongy mesophyll is also variable having two to six layers of parenchyma cells. Combined, these factors form a leaf blade that is not uniformly thick.

The hollow petiole is round in transverse section near the stem and becomes dorsally grooved near the blade. Marginal strips of collenchyma run its length and provide support. The petiole contains thirteen vascular bundles arranged circularly in pairs with the ventral most bundle unpaired. In the transition zone between petiole and blade, the vascular bundles coalesce before they divide and branch into the five midribs.

In the proximal region of the leaf blade, the central midrib contains seven vascular bundles. The bundles are arranged in an upturned crescent. A large bundle lies lowermost and is flanked on each side by

three bundles that decrease in size. Advancing distally through the blade, the smaller uppermost bundles successively branch off into the secondary or lateral veins. The dorsal surface of each midrib is also slightly raised by a ridge of underlying collenchyma cells.

The vascular bundles of *C. pepo* are distinctively bicolateral. Bundles in the stems, petioles and midribs have two substantial regions of phloem, one outside the xylem and the other inside. Between the xylem and both regions of phloem are zones of cambium; the outer cambium zone, however, is larger and more active then the inner. In the minor leaf veins the bicolateral bundles are reduced to a single upper sieve element with companion cell, a file of two or three tracheids, and two lower sieve elements with companion cells (Turgeon et al., 1975).

The entire leaf surface, blade and petiole, are covered with numerous trichomes. They impart a prickly or harsh texture to the leaves which is characteristic of *C. pepo*. The lower blade surface has the higher density of trichomes, averaging 340 mm^{-2}. Inamdar *et al* (1990) has identified five glandular and six eglandular trichome types in the genus *Cucurbita* which can be used to distinguish it from other Cucurbitaceae. They have also described three stoma types for *Cucurbita*: anomocytic, paracytic and stoma with a single subsidiary cell.

Leaf Development

Leaf development in *C. pepo* has not been thoroughly investigated but can be assumed to be similar to other dicotyledons. Fahn (1990) divides dicot leaf development into five overlapping stages: initiation, early differentiation, leaf axis development, lamina development, and tissue

histogenesis. This outline forms the basis for the following discussion with additions on *C. pepo* where available.

Initiation is the formation of a leaf primordium, a localized outgrowth on the side of the shoot apex. It results from periclinal divisions in either the inner tunica or outer corpus layers and occurs either immediately adjacent to the shoot apex or further down the apical cone. The primordium is raised into a leaf buttress by the continual division of sub-surface cells. Surface tunica cells also divide anticlinally giving rise to a covering protoderm which continues to divide as the buttress is raised above the shoot apex.

C. pepo's main axis is a sympodium (Whitaker and Davis, 1962). At each node an axillary bud continues the main axis by displacing the terminal bud. A single leaf buttress is raised on the outer edge of the displaced terminal bud. The inner edge is the site of flower formation. Because displacement of the axis shifts from side to side an alternating leaf pattern is produced.

Continued apical growth of the leaf buttress forms an elongated cone-like structure which is flattened on its adaxial side. As the buttress grows it differentiates and consists of an outer protoderm layer, an inner mass of ground meristem, and a procambial strand that extends acropetally from the procambium trace of the stem. Maturation occurs basilpetally and continued elongation in many species shifts from apical to intercalary growth. The rate of growth is often greater on the abaxial side causing the structure to bend over the apical meristem as it increases in length. Elongation of the buttress establishes the leaf axis

which in dicots usually precedes lamina development.

Once the leaf axis attains a certain length, two small lateral outgrowths appear on either side of the axis. These constitute the marginal meristems which establish the cell layers in the developing lamina. The marginal meristems are composed of marginal and sub-marginal initials. The marginal initials are located on the outer edges of the lamina; they divide anticlinally and add cells to the upper and lower protoderm layers. The sub-marginal initials are interior to the marginal initials; they divide in various planes and give rise to three types of internal layers: adaxial, middle and abaxial. Activity of the marginal meristem is replaced by cells within the lamina layers; they function as plate meristems, dividing anticlinally to complete lamina expansion. The lobed leaves of *C. pepo* result from differential growth of the marginal meristem while its petiole results from the repression of growth (Troll, 1939).

Differentiation of leaf tissues follows different patterns in different species. In general, the protoderm gives rise to epidermis, the adaxial layers form the palisade mesophyll, and both the middle and abaxial layers contribute to the spongy mesophyll. Development of the vascular system in the midrib precedes lamina expansion. Vascular bundles, their sheaths and supporting structures arise from the procambial strands that develop during buttress elongation. Development of the large, small, and minor lateral veins occurs during lamina expansion and precedes mesophyll differentiation. They arise from procambial traces that originate in either expanding middle or abaxial layers.

In *C. pepo* lateral vein development occurs in the abaxial layer, begins at the leaf tip, and moves basipetally through the blade (Turgeon and Webb, 1976). Maturation of phloem and xylem begins in upper half of each vascular bundle and moves rapidly into the lower half. Transition of the leaf from an importing to an exporting organ occurs after the phloem achieves structural maturity (Turgeon and Webb 1975). Although sucrose synthesis occurs throughout leaf development, its export does not begin until the areolas are delimited by the minor veins .

Enlargement, division and differentiation of lamina cells continues throughout the growth of the young leaf. Lateral vein formation divides the developing lamina into pockets of expanding cell layers. Leaf growth is anisotropic; different regions of the leaf expand at different rates and directions in response to genetic and environmental factors. The palisade mesophyll is the last tissue to cease meristematic activity, it continues to grow and divide well after the spongy mesophyll and epidermis. The basic number of cells and the fundamental leaf structure are established before the leaf unfolds; a set number of rounds of division determine final leaf size.

Genetic Factors Affecting Leaf Pattern and Development.

A cursory inspection of published gene lists reveals that leaf pattern and development are genetically determined. Hundreds of mutants have been characterized that influence leaf size, shape, phyllotaxis, serration, pigmentation, and the formation of stomata and trichomes (O'Brien, 1990). In *C. pepo*, two genes have been reported to affect leaf structure. The dominant gene mottled, *M* , produces silver gray areas in the axils of

leaf veins as a result of localized air spaces between the upper epidermis and palisade mesophyll (Scarchuk, 1954; Scarchuk and Lent, 1965). The recessive gene rosette, *ro* , imparts a spiraled appearance to the lower leaf lobes (Mains, 1950); unfortunately, genetic stock is not available for comparison to the fused vein trait (Robinson and Hutton, 1992). *C. pepo* leaf lobing is also under genetic control; available cultivars display a range of stable lobe phenotypes and a single lobing gene has been reported in *C. maxima* and *C. ecuadorensis* (Dyutin, 1980; Herrington and Brown, 1988).

Though individual genes specify discrete structures, and multiple genetic systems govern size or number, knowledge of their role in a coordinated developmental system is largely unknown in plants (Gottlieb and Ford, 1987). Nevertheless, Sachs (1991) maintains that plant development is epigenetic; the patterns inherent within undifferentiated cells are induced, determined and maintained through a cascade of decreasing developmental options in response to competing molecular signals. Plant growth regulators, particularly auxins and cytokinins, have been implicated as the primary signals that induce and/or repress developmental gene activity.

Studies with the leaf mutants of *Pisum sativum* suggest that the leaf development cascade results from repeated compartmentalization (Marx, 1987). In a manner analogous to early animal development in *Caenorhabitis* and *Drosophila*, homeotic-like genes define three major leaf compartments: basal, middle and terminal; within which specific genes operate to cause the formation of stipules, leaflets and tendrils. Combinations of various

P. sativum mutants transform tissues of one compartment into those of another, for example, placing tendrils where leaflets should be. The mutants alter the size, growth and subdivision of leaf meristems by changing both the number and plane of cell division (Meicenheimer *et al*, 1983; Young, 1983; Gould *et al*, 1986). Similar studies of genetic regulation of leaf histogenesis, anatomy, and morphology are lacking in other species. However, a connection between the genetic control of cell division and structural development has been demonstrated for the formation of ray florets in *Layia discoidea* (Gottlieb and Ford, 1987) the hooded trait in *Hordeum vulgare* (Stebbins and Yagil, 1966) and fruit size in *Capsicum annuum* (Sinnott and Kaiser, 1934; Kaiser, 1935).

Part II: Gametophytic Section

Angiosperm Life Cycle

The angiosperm life cycle alternates between a prominent diploid sporophyte generation and an inconspicuous haploid gametophyte generation. By meiosis, the sporophyte produces megaspores and microspores in the ovaries and anthers of its flowers. The megaspores grow, divide mitotically, and differentiate into clusters of cells forming the female gametophytes or embryo sacs. Likewise, the microspores grow, divide and differentiate into male gametophytes or pollen. The gametophytes produce gametes, egg and sperm, which through pollination and fertilization fuse to form new sporophyte zygotes. The zygotes develop into seed which upon dispersal and germination grow into new sporophytic plants. Although small and short-lived, gametophytes facilitate sexual reproduction in

plants. They are the bridge that permits the recombination of genes by connecting meiosis with fertilization.

Tenets of Gametophytic Selection

The concept of gametophytic selection asserts that selection for sporophytic traits occurs not only during the sporophyte generation but during the gametophyte generation as well. It is based on three assumptions: 1) that pollen composition and function are directed, in part, by its haploid gene complement, 2) an overlap in gene expression exists between the sporophyte and gametophyte generations, and 3) that the processes of plant reproduction are selective and competitive (Mulcahy, 1975; Zamir, 1983). Various investigations over the last seventy years have substantiated these assumptions.

Gametophyte Gene Expression

Brink and MacGillivary (1924) first observed gametophytic gene expression in corn, *Zea mays*. Using a simple iodine stain, they found a 1:1 ratio for amylose expression among pollen from plants heterozygous for the recessive waxy gene. Their finding demonstrated Mendel's law of allele segregation and revealed a gametic origin for starch composition in pollen.

The discovery of gametophytic self-incompatibility in *Nicotiana sanderae* by East and Mangelsdorf (1925) was another early indication of gametophytic gene expression. Eventually detected throughout the angiosperms, this form of incompatibility is determined by the genotype of the pollen and not the plant that produces it. Characteristically, crosses between plants differing in one incompatibility allele (S_1S_2 female

x S_1S_3 male) exhibit selective transmission of only the different allele (S_3). Examinations of *Onenothera*, *Lycopersicon*, and *Lilium* have shown that recognition of incompatible pollen can occur at the stigmatic surface, within the style, or at the ovary. (de Nettancourt 1977). The implication is that gametophytic gene activity is involved with a variety of gametophytic functions.

Analysis of pollen wall development and composition during the late sixties supplied indirect evidence of gametophytic gene expression. Although the outer pollen wall, the exine, relies heavily on the paternal tapetum for its formation, the inner wall, the intine, is composed of gametophytic cell products (Heslop-Harrison, 1971). The intine is a matrix of hemicelluloses, pectic materials and imbedded proteins. Concentrated near apertures are hydrolytic enzymes involved with germination, stigma penetration, and early pollen tube nutrition. Intine proteins are also known to mediate interspecific recognition (Knox *et al.*, 1972) and gametophytic incompatibility (Heslop-Harrison *et al.*, 1973).

Electrophoretic investigations verified that several pollen proteins are derived from gametophytic genes. Dimeric enzymes in the pollen of tomato, *Lycopersicon esculentum*, (Tanksley *et al.*, 1981) corn, *Zea mays*, (Sari-Gorla *et al.*, 1983), squash, *Cucurbita pepo*, (Gay *et al.*, 1986) and barley, *Hordeum vulgare*, (Pedersen, 1988), were examined. Banding patterns of pollen extracts from heterozygous plants lacked heterodimer bands, indicating a gametophytic origin for the enzymes. Similarly, a refined electrophoretic technique, microelectrophoresis, was applied to individual pollen grains from interspecific *Cucurbita* hybrids; a diverse

set of banding patterns also confirmed the presence of gametophytic gene activity (Mulcahy *et al.*, 1979; Miller and Mulcahy, 1983).

Genetic Overlap In Gametophyte And Sporophyte Generations

The electrophoretic evidence also supported the genetic overlap assumption. Tanksley and coworkers (1981) compared expression of thirty enzymes in both sporophytic and gametophytic tomato tissues. Sixty percent of the enzymes were present in both generations, 37 percent were sporophyte specific, and 3 percent were found only in pollen. Additional electrophoretic studies, have found similar patterns of gene expression in corn (Sari-Gorla *et al.*, 1986), barley (Pederson *et al.*, 1987) and *Populus* species (Rajora and Zsuffa, 1986). DNA-mRNA hybridization studies in *Tradescantia* have also shown that a majority of the genes transcribed by the male gametophyte are expressed by the sporophyte (Willing and Mascarenhas, 1984; Willing *et al.*, 1984).

Genetic overlap has been demonstrated indirectly by comparison of phenotypic expression. Evans and coworkers (1988), detected significant correlations between the levels of both linoleic and linolenic acids in the pollen and seed lipids of oilseed rape, *Brassica napus*. Superior seed weight and seedling height have been associated with rapid pollen tube growth rate in corn (Mulcahy, 1974; Ottaviano *et al.*, 1980). Moreover, gametophyte and sporophyte generations have shown parallel tolerance to *Helminthosporium maydis* toxin in corn (Laughnan and Gabay, 1973), to copper in *Mimulus guttatus*, to zinc in *Silene* species (Searcy and Mulcahy, 1985) to high temperature in corn (Herrero and Johnson, 1980) to low temperature in melon, *Cucumis melo*, (Hutton, 1988) and to the herbicide

ethofumesate in sugar beet, *Beta vulgaris*, (Smith and Moser, 1985). Although phenotypic expression was examined, it was assumed that the traits were governed by the same genes in both generations. These studies also revealed that gametophytic activity is not limited to specific reproductive functions.

Selective And Competitive Reproduction

Accumulated evidence has shown that the maternal processes of plant reproduction are selective. De Nettancourt's (1977) review of plant incompatibility describes a multi-layered selection system. Flower morphology, stigmatic recognition, pistil environment and embryo rejection act in concert to screen male gametophytes. Mixed pollen experiments with corn (Pfahler, 1965, 1967), onion, *Allium cepa*, (Currah, 1983), and radish, *Raphanus sativus*, (Marshall and Ellstrand, 1986) have shown that this selection system extends beyond the determination of compatibility. Pollen mixtures from two or more compatible lines were applied to individual stigmas. Maternal choice resulted in uneven fertilization; genetically different stigmas preferred different pollen sources. Selective abortion of fruit with low seed set has also been suggested as a maternal mechanism to maximize selection and improve offspring quality (Lee, 1984). Seed set has been associated with pollen load and offspring quality (Jennings and Topham, 1971; Mulcahy *et al.*, 1983; Schemske and Fenster, 1983; Shore and Barrett, 1984; Stephenson and Windsor, 1986; Windsor *et al.*, 1987). Presumably, a high pollen load produces strong pollen competition, maximizes gametophytic selection and leads to improved offspring quality.

An extensive examination of pollen tube growth, fertilization success and effects on sporophyte expression in corn has demonstrated the presence of pollen competition and the agronomic potential of gametophytic selection. Johnson and Mulcahy (1978) clarified the work of Jones (1928) and Pfahler (1965, 1967) and showed that pollen tube growth rate is dependent on the gametophyte's genotype. It was recognized that fast growing pollen has a selective advantage in fertilizing basal embryo sacs on developing ears (Mulcahy, 1974). Ottaviano and coworkers (1983) used an open pollinated corn line to develop inbreds based on seed position. Basal lines had faster pollen tube growth rates *in vitro*, displayed superior growth and yield, and combined to form better hybrids than the apical lines. This connection between enhanced sporophyte vigor and gametophytic competition has also been reported for *Dianthus chinensis* (Mulcahy and Mulcahy, 1975) and *Petunia hybrida* (Mulcahy *et al.*, 1975).

Biological Significance

The existence of gametophytic gene expression, genetic overlap, and selective and competitive reproduction has important biological significance. Gametophytes can not be viewed as neutral parties in the reproductive process (Mulcahy, 1975). The numerous pollen grains deposited on a stigma are a population of haploid individuals competing to fertilize a relatively few number of ovules (Zamir, 1983). Mulcahy (1979) contends that the strength and immediacy of gametophytic selection was responsible, in part, for the evolution of angiosperms. An ascent of the phylogenetic tree finds increasing rates of pollen germination and tube growth, which are the imprints of this evolutionary process (Hoekstra, 1983).

Gametophytic selection changes allele frequencies in the sporophyte generation. Traits that enhance gametophyte performance will increase at the expense of those that diminish performance. Using 34 recessive endosperm mutants in corn, Ottaviano and coworkers (1988) demonstrated the influence of gametophytic selection on allele frequency. Eleven of the mutants produced distorted F_2 inheritance ratios with lower than expected mutant recovery; they also exhibited poor pollen quality indicating gametophytic selection. Five mutants displayed diminishing mutant recovery from ear apex to base revealing competition during pollen tube growth. Distortion of inheritance ratios and accompanying reductions in pollen fitness have also been demonstrated for the *Fusarium* resistant gene in tomato (Keder *et al.*, 1967 and Rabinowitch *et al.*, 1978) and the stringless trait in garden pea, *Pisum sativum* (McGee and Baggett, 1992a and 1992b).

Manipulation of gametophytic selection to influence inheritance was first demonstrated by Buchholz and coworkers in 1930 and 1932 using *Datura stramonium*. Not until recently, however, has gametophytic selection been employed for crop improvement. Using a cold temperature pollination technique, Zamir and coworkers (1981, 1982, 1987) preferentially transferred genetic material from the cold tolerant *Lycopersicon hirsutum* to the cultivated tomato and achieved improved root growth at low temperatures. Similarly, heat tolerance from *Gossypium barbadense* has been transferred to cultivated cotton, *Gossypium hirsutum*, by heat-treating pollen before pollination (Rodriguez-Garay and Barrow, 1988). Sacher and coworkers (1983), by pollinating under high salt conditions, significantly

increased the salt tolerance of F_2 populations derived from the hybrid of intolerant *L. esculentum* and tolerant *Solanum pennellii*. Improved uniformity and shifts in plant height, branch number and leaf size were obtained in populations of alfalfa, *Medicago sativa*, after applying a variety of stresses (drying, aging, freezing and high and low temperatures) during microsporogenesis, post anthesis, and during pollen tube growth (Mulinix and Iezzoni, 1988) Crop improvement through gametophytic selection is an attractive concept. Provided desirable sporophytic traits are expressed in the gametophyte, it permits the screening of many haploid individuals in a short period of time and in a small amount of space.

CHAPTER III

Morphology and Anatomy of the Fused Vein Trait

Abstract

The morphology and anatomy of the fused vein trait were characterized in *Cucurbita pepo* L. using the inbred NH2405 as the phenotypic archetype. Fused vein expression was also studied in a partially fused line, NH7210, and among fused vein segregants in F_2, F_3, and backcross populations generated between NH2405 and the normal inbred NH614.

Morphological analysis showed that the trait is characterized by a partial fusion of the five primary leaf veins. Fusion begins at the distal point of the petiole and extends along the central vein. Branching of the veins is delayed and there is a reduction of the intraveinal leaf blade. Consequently, the dorsal leaf surface appears puckered or wrinkled. Depending on genetic background, fused vein expression starts at the fourth to tenth leaf stage and continues throughout vegetative growth. The extent of fusion increases with leaf number but stabilizes by the twentieth leaf stage. The maximum extent of fusion also varies with genetic background from slight (1-5 cm) to pronounced (15-20 cm).

Anatomical comparison of normal and fused vein leaves revealed different vascular patterns in the transition zone between petiole and leaf blade. In normal leaves, the vascular bundles of the petiole enlarge and coalesce to form a vascular crescent. The crescent reorganizes and

diverges as large vascular columns and pairs of smaller flanking vascular bundles into each vein. In contrast, two cycles of enlargement, coalescence, and dispersal occur in fused vein leaves. In the first cycle, a bipolar vascular shaft forms in the transition zone. It reorganizes and diverges, forming the vasculature of the central vein and a partial petiole-like ring of vascular bundles in the upper transition zone. Four of these bundles become the vascular columns in the inner and outer laterals. In the second cycle, the remaining bundles, after a brief period of division, reconverge to form two secondary shafts of vascular tissue. These shafts reorganize and generate the pairs of flanking bundles that diverge into the leaf veins.

Introduction

As a species, *Cucurbita pepo* L. displays considerable variation in both vegetative and reproductive structures (Whitaker and Davis, 1962). Mature plants range from compact single stem bushes to rambling multi-stem vines. Leaves show subtle variation in lobe depth, sinus width, trichome harshness, and surface mottling. Fruits vary greatly in color, shape, size and texture. Indeed, there are nine distinct cultivar groups based on fruit type alone (Paris, 1989).

The requirements of large-scale mechanized agricultural, however, have narrowed the vegetative appearance of leading *C. pepo* cultivars. Private and public breeders have emphasized bush and semi-bush habits with efficient non-mottled, shallow-lobed leaves. An increasing number of cultivars with a variety of fruit types and this vegetative form are available commercially. Although they offer cultural and economic advantages, their proliferation presents an added challenge to the already difficult maintenance of *C. pepo* cultivars.

A cultivar's purity is continually challenged by natural mutation, accidental contamination, and uncontrolled outcrossing. Rouging, the removal of offtypes, is a routine stock and commercial seed production method to maintain purity and prevent subsequent contamination. For cross-pollinated *C. pepo* cultivars, timely detection of offtypes has always been difficult. Rouging occurs prior to flowering among juvenile plants where offtypes exhibit only subtle vegetative differences. Consequently, the trend of increasing vegetative similarity among leading cultivars further impedes cultivar maintenance.

New challenges to *C. pepo* purity protection especially threaten the expanded use of hull-less seeded cultivars for snack and oil seed crops. Hull-less seeded cultivars are morphologically similar to many hulled pumpkins and are maintained and produced in the same agricultural region. Currently, their seed acreage is comparatively small and adequate isolation is difficult to obtain. The hull-less trait is determined by a maternally expressed recessive gene, n (Stuart, 1983). As a result, outcrossing by similar hulled genotypes can remain undetected for a generation allowing widespread contamination of stock seed. The need for frequent and costly renewal of stock through hand-pollinated trueline selection has and will continue to hinder the use of hull-less seeded cultivars.

A new mutant leaf form, fused vein, has potential as a rouging marker for hull-less seeded *C. pepo* cultivars. The trait was first observed by Dr. J. Brent Loy in 1987 among segregating material descending from a cross between "Trickijack" and "Minijack". It has since been fixed in two hull-less seeded inbred lines, NH2405 and NH7210. In both lines, it is expressed prior to flowering and imparts a distinctive, conspicuous, leaf morphology. The trait is rare and inherited stably. Limiting its use to hull-less material would provide broad outcross detection from hulled contaminants.

The purpose of this study was to characterize the morphology and anatomy of the fused vein trait. Using selected breeding lines, the extent and stability of the fused vein phenotype were determined.

Materials and Methods

Plant Material. The *Cucurbita pepo* L. inbred line NH2405 served as the archetype for the fused vein phenotype. The related partially fused line NH7210 provided additional information on expression (Figure 1.1). Both lines were compared to the normal phenotype displayed by the unrelated inbred NH614. The three lines were supplied by Dr. J. Brent Loy, Department of Plant Biology, UNH, Durham, NH. Fused vein segregants in reciprocal F_2, F_3 and backcross populations between NH2405 and NH614, generated by controlled hand pollination for inheritance studies (see Chapter IV), were also examined.

Morphological Study. Observations of fused vein and normal plants were made during the summers of 1990, 1991 and 1992 at the Woodman Horticultural Farm among field plots used for inheritance and pollen competition studies (see Chapters IV and V). Although individual plots experienced different environmental and cultural conditions, they all received ample fertilizer, supplemental irrigation, and excellent pest and non-chemical weed control. Lines in all plots were planted in adjacent rows six feet apart with two feet between plants. In all cases, plant growth and development were typical for field grown *C. pepo*. Each plant in every plot was examined throughout its growth for the initiation of fused vein leaves and the extent of vein fusion. Additionally, in the 1991 pollen competition plot, measurements were made of 10 randomly selected NH2405 plants. The length of vein fusion from the distal end of the petiole to divergence of the inner lateral veins was measured for the first 20 leaves borne on the main stem.

Anatomical Study. Tissue samples of NH2405 and NH614 were collected from randomly selected field grown plants at the Woodman Horticultural Farm, Durham, NH in mid August 1991. Samples consisted of the upper 3-5 cm of either a main or lateral stem, including all attached leaves and flower buds. They were preserved in Carnoy's Solution (75% alcohol 25% acetic acid) and stored for later processing and observation in the Winter of 1991-92.

Standard histological procedures were employed as outlined by Jensen (1962). Leaves approximately 1 cm in length, with clearly visible veination patterns, were selected and dissected from the tissue samples. They were dehydrated in an ethyl and tertiary butyl alcohol series, imbedded in paraffin, and serially cross sectioned at 14 μ using a rotary microtome. Five good series were obtained for each genotype, affixed to glass slides using Haupt's adhesive, deparaffinized, stained with safranin and fast green, and mounted with Canadian Balsam. Both normal and fused vein sections were compared microscopically for differences in vein structure and vascular distribution.

Results and Discussion

Morphological Description. The fused vein phenotype of NH2405 is characterized by a partial fusion of the five primary leaf veins: a central vein and two outer laterals, and two inner laterals (Figure 3.1 and 3.2). Fusion begins at the distal point of the petiole and extends to more than half the length of the leaf blade. Initially, all five veins are united; the outer laterals diverge first into the lower leaf lobes, followed by the inner laterals into the upper lobes. Although the veins are joined, they are visibly distinct.

Production of fused vein leaves begins with the fourth leaf stage and continues throughout vegetative growth. The extent of fusion varies with leaf number (Table 3.1); it is moderate in early leaves (5 cm), is

Table 3.1. Mean length of vein fusion by leaf for ten NH2405 plants.

Leaf Number	Length of vein fusion (cm)		Standard Deviation
	Mean	Range	
1	0.00	*	*
2	0.00	*	*
3	0.00	*	*
4	4.79	3.5 - 5.6	.623
5	5.07	4.0 - 6.3	.648
6	7.21	5.9 - 8.2	.651
7	8.46	7.6 - 9.4	.562
8	8.14	7.1 - 9.1	.595
9	9.46	8.2 - 10.8	.701
10	10.38	9.3 - 11.7	.667
11	10.90	9.7 - 11.7	.603
12	11.81	10.8 - 12.5	.552
13	13.20	12.3 - 14.5	.637
14	14.37	13.3 - 15.6	.645
15	14.89	13.6 - 15.7	.623
16	15.14	14.0 - 16.1	.596
17	15.37	14.2 - 16.7	.684
18	15.10	13.8 - 16.8	.783
19	15.52	14.5 - 16.3	.565
20	14.88	13.9 - 16.1	.632

Figure 3.1. Fused vein leaf, ventral view.

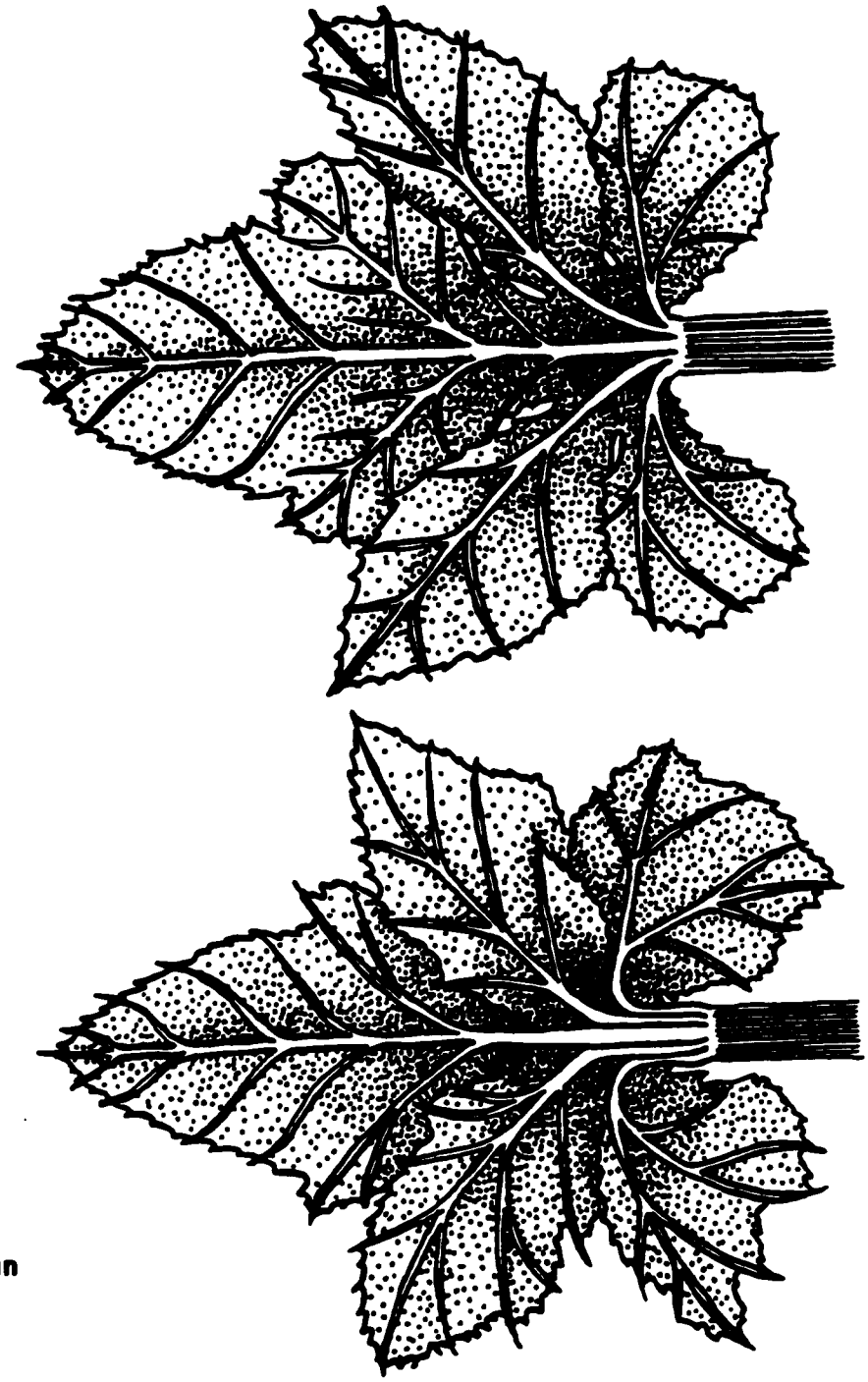


Figure 3.2. Comparison of normal and fused vein leaf venation.

pronounced by the tenth leaf stage (10 cm), and becomes stabilized and extensive by the sixteenth leaf stage (15 cm). With extensive fusion, several secondary veins are fused to the central and primary veins. The intraveinal leaf blade is also reduced and the dorsal leaf surface appears puckered or wrinkled (Figure 3.3). Consequently, the fused vein trait confers a distinctive appearance to juvenile and mature plants when compared to the normal phenotype (Figure 3.4).

The pattern of vein fusion was the same for all NH2405 plants regardless of the plot examined. Observation of NH7210 and fused vein segregants in F_2 and F_3 populations, however, revealed that fused vein morphology varies with genetic background. In NH7210, vein fusion begins similarly at the fourth leaf stage and increases in magnitude but it is never as extensive as in NH2405. In F_2 populations, the onset of fused vein leaf production and the extent of vein fusion also varied between individual fused vein plants. Onset of production ranged from the fourth to the tenth leaf stage and the maximum extent of fusion varied from slight (5 cm) to pronounced (20 cm). The greatest vein fusion occurred in plants with the earliest onset of fused leaves and the deepest leaf lobes. Similar variation existed between F_3 lines, however, within an individual line plants were all phenotypically similar. To facilitate rouging, lines with early and pronounced expression should be selected.

There were no other unusual morphologies associated with this trait. Stem fasciation and female flower cohesion, found in some *C. pepo* lines, were both absent. Dr. Loy has noted that the subtending leaf of pistillate flowers in NH2405 is occasionally fused to the flower's peduncle and ovary, however, this has also been observed in other normal inbred lines.

Figure 3.3. Fused vein leaf, dorsal view.

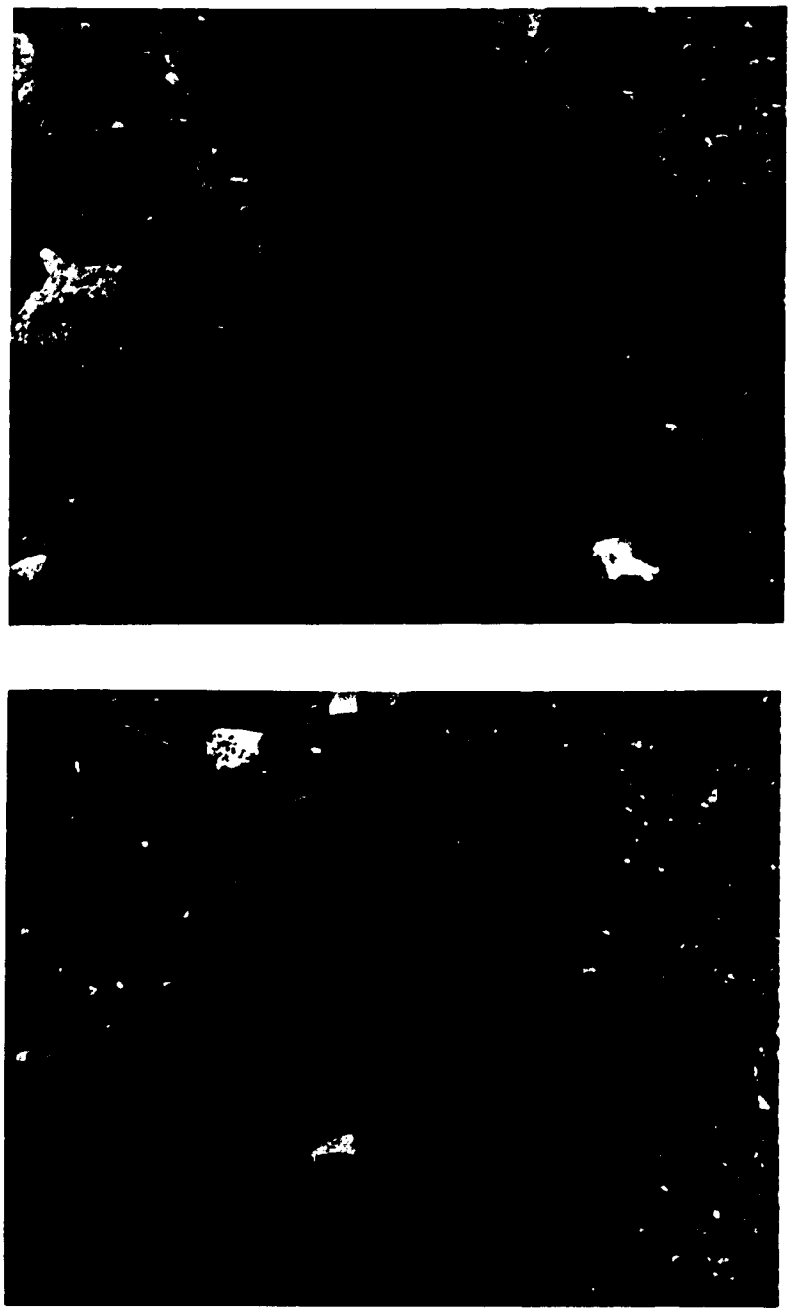
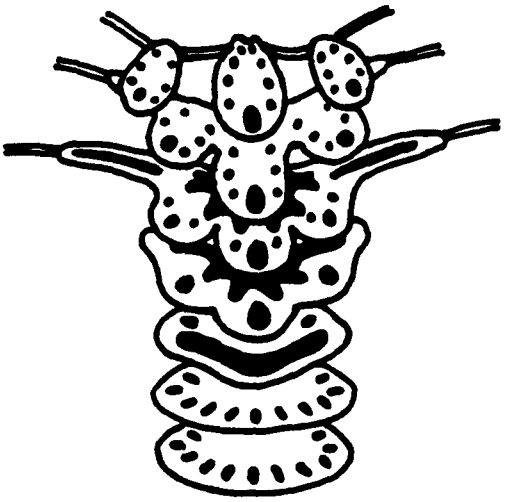
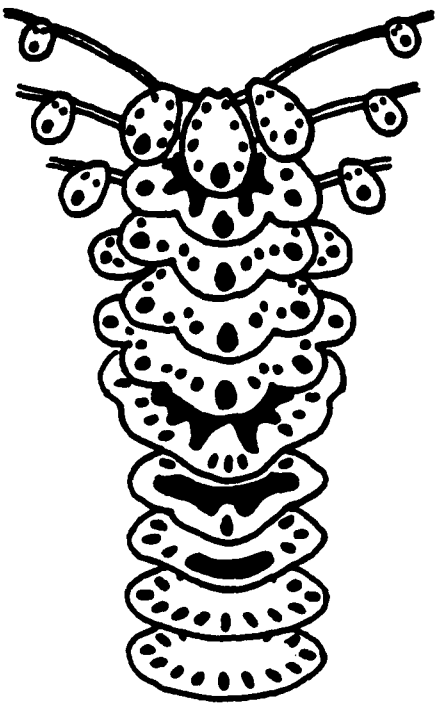


Figure 3.4. Comparison of normal and fused vein juvenile plants.

Anatomical Description. The sectioned 1 cm length leaves of both normal and fused vein genotypes were miniature versions of fully expanded leaves, complete in form and organization but still in the processes of lamina expansion and tissue histogenesis. The leaf blades were the least developed, consisting of protoderm, adaxial, middle, and abaxial layers. There was no differentiation of palisade or spongy mesophyll cells, or stomata or trichomes. Minor vein development had begun with procambial traces appearing in swellings of the middle leaf blade layer. In contrast, development of the vasculature in the petioles and primary and secondary veins was extensive. The arrangement and bicolateral organization of the vascular bundles were evident as were protophloem, protoxylem and bundle sheaf cells. Unlike the leaf blade, trichomes were present on their surfaces and strands of presumptive collenchyma were observed; the absence of red safranin stain, however, indicated no lignification had occurred. Comparison of normal and fused vein leaf sections revealed a similar arrangement of vascular tissue in their petioles. Near the stem, there are thirteen vascular bundles arranged circularly in pairs with the ventral most bundle unpaired. Near the blade, pairing of the bundles ceases and two to three of the dorsal most bundles divide into two. As the bundles enter the transition zone between petiole and leaf blade, they enlarge and coalesce before separating into the five primary leaf veins. The pattern of coalescence and divergence, however, and the final number of bundles in each vein differs between normal and fused vein leaves, as schematically represented in Figure 3.5.



Normal



Fused Vein

Figure 3.5. Schematic comparison of vascular bundle arrangement in the transition zone of normal and fused vein leaves (ventral view).

In normal leaves, the vascular bundles in the ventral half of the petiole expand and merge, forming a crescent of vascular tissue as they enter the transition zone (Figures 3.6-3.8). Invagination of the epidermis and cortex defines and eventually separates the individual leaf veins. As flanking bundles enlarge and join the crescent, the crescent reorganizes. Three distinct vascular columns develop within the lower and outer portion of the crescent and then diverge into the central and inner lateral veins. The adjoined flanking bundles also form columns that diverge abruptly into the two outer lateral veins. As the five columns diverge and assume ventral positions in each vein, the vascular crescent is partially split. Consolidation of the remaining vascular tissue and the convergence of additional dorsal bundles forms a stelliform vascular shaft, occupying the upper half of the transition zone. From the edges of the shaft, individual bundles diverge in pairs into the leaf veins. With completion of invagination, the central and lateral veins contain a large vascular column flanked by six or eight smaller vascular bundles, respectively. As each leaf vein branches into pairs of successive secondary veins, the dorsal most bundles diverge into the secondaries until, finally, the vascular columns themselves divide and diverge into the distal most secondaries.

In the fused vein line, the vascular bundles do not completely coalesce to form a crescent (Figures 3.9-3.13). The ventral-most bundle of the petiole remains partially distinct, fringed by parenchyma cells. As invagination begins, this bundle diverges into the central leaf vein by itself, leaving a bipolar shaft of vascular tissue. As flanking

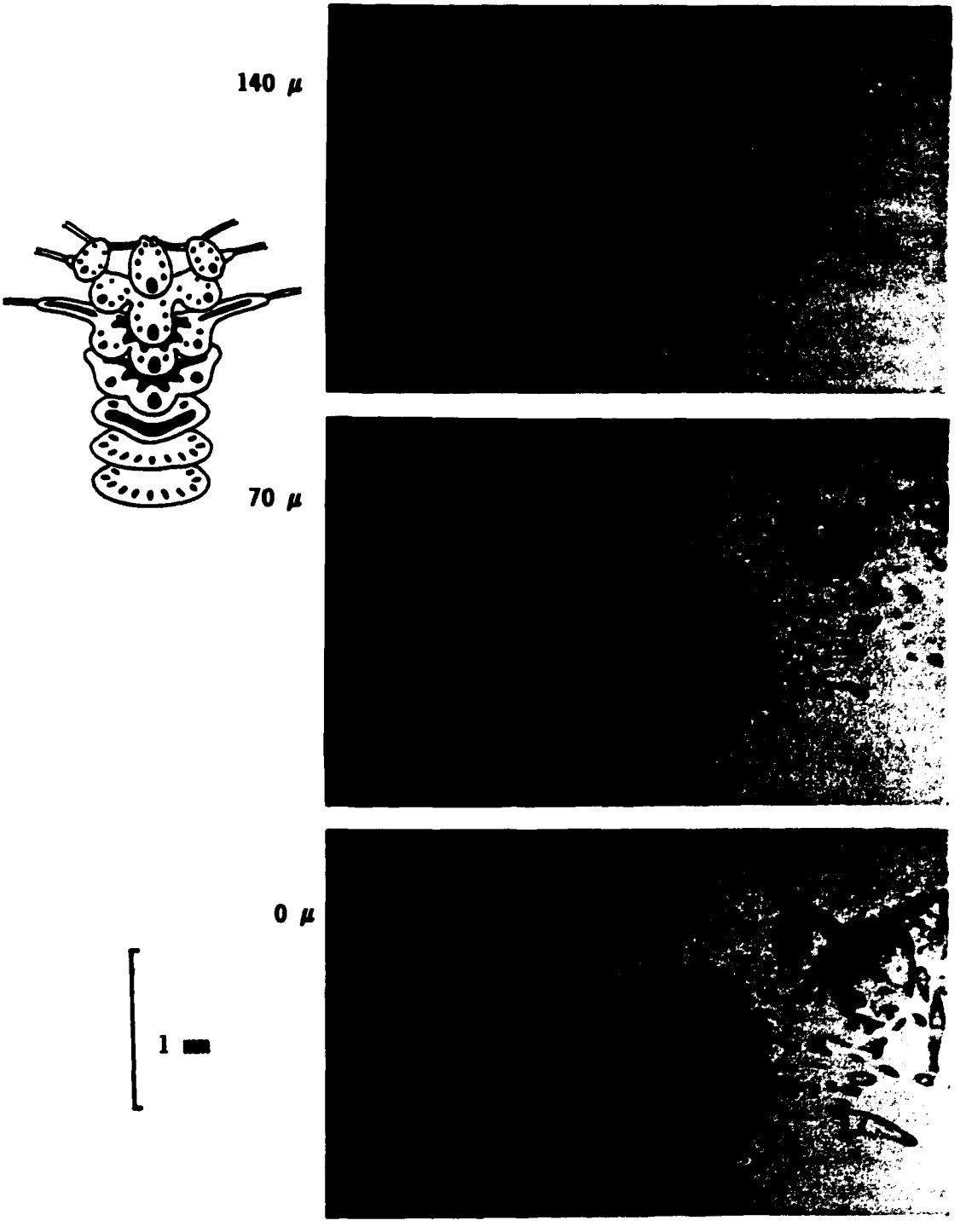


Figure 3.6. Normal leaf serial cross sections; 0, 70 and 140 microns from the petiole/leaf blade junction.

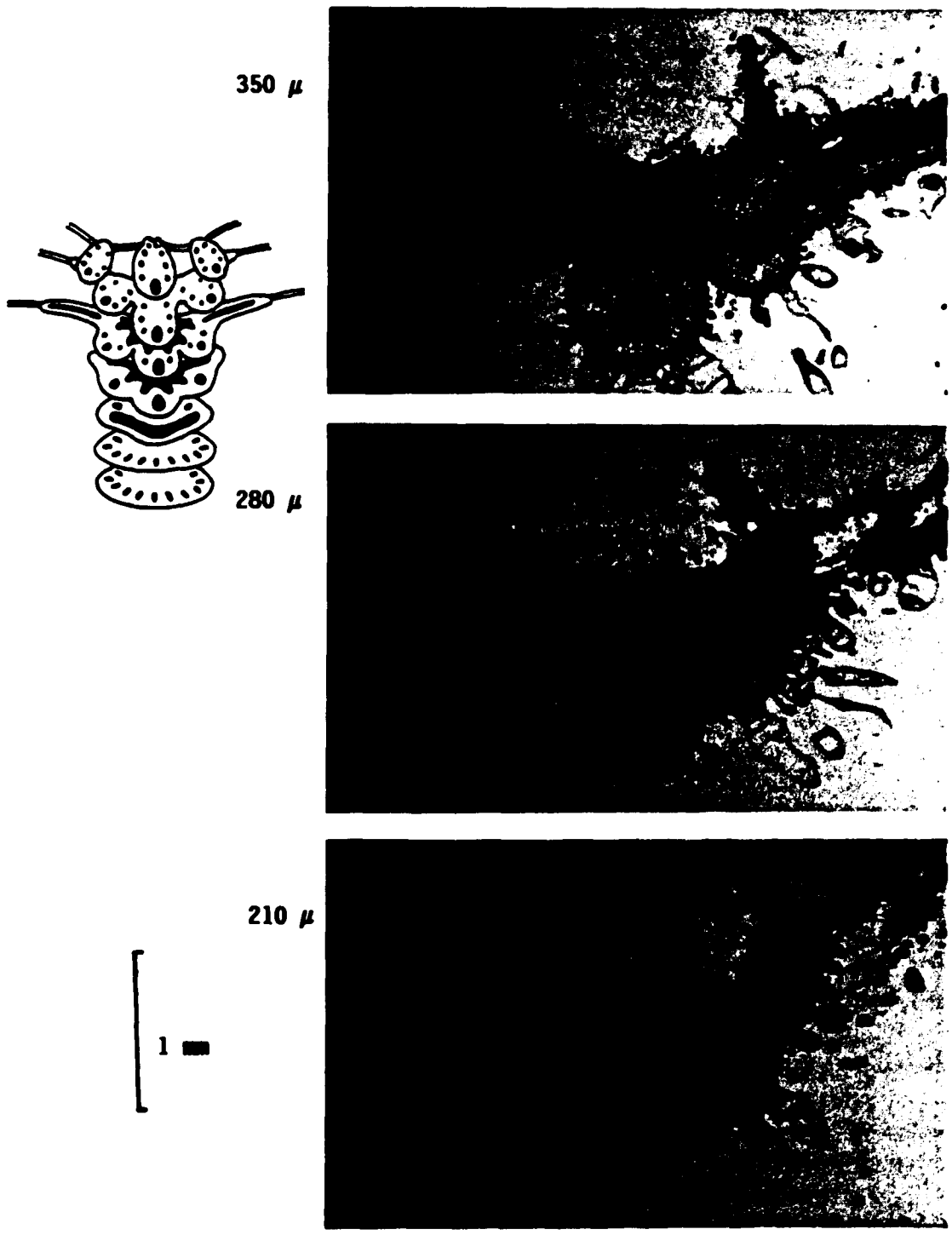


Figure 3.7. Normal leaf serial cross sections; 210, 280 and 350 microns from the petiole/leaf blade junction.

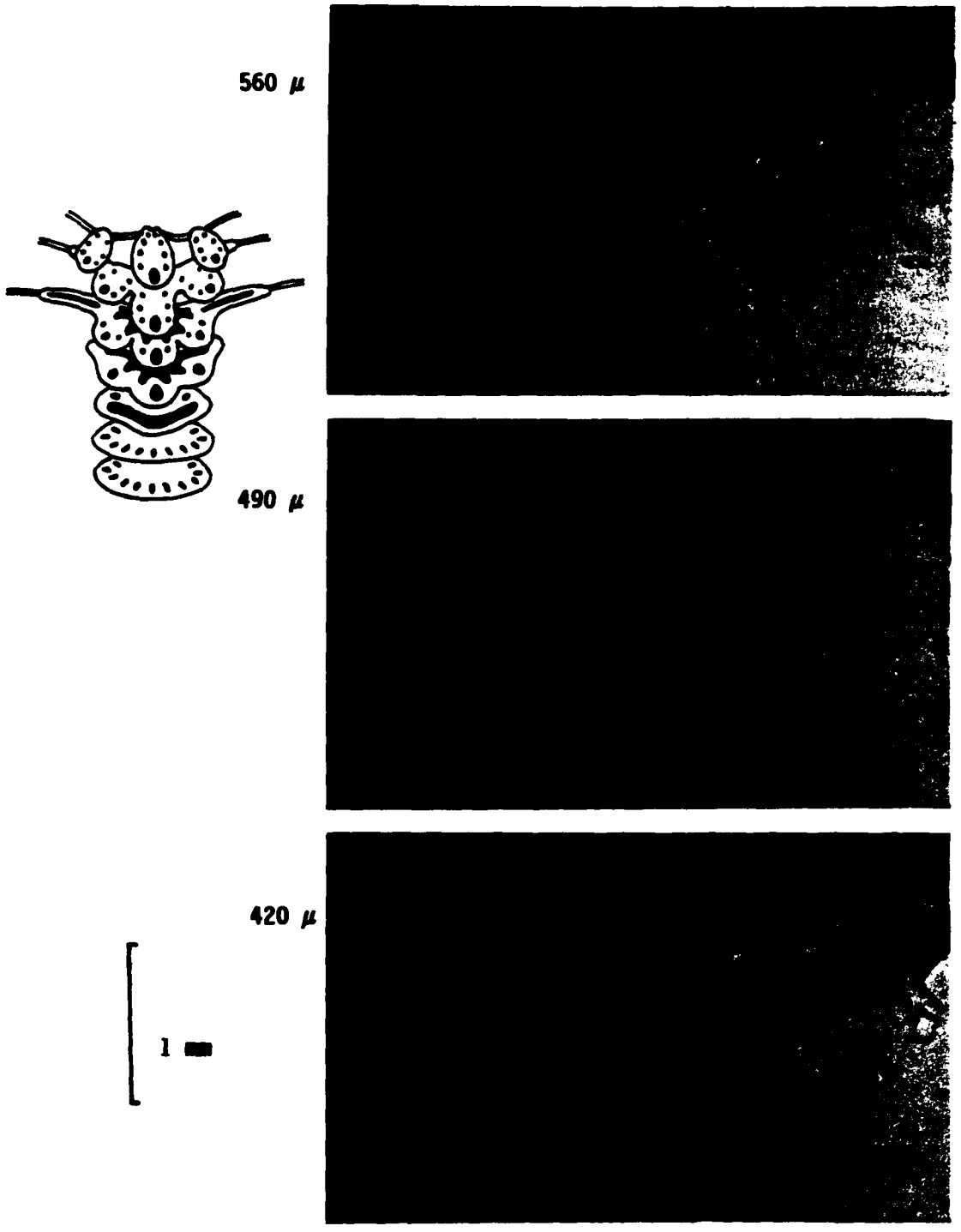


Figure 3.8. Normal leaf serial cross sections; 420, 490 and 560 microns from the petiole/leaf blade junction.

bundles merge with the shaft, the shaft gravitates to the dorsal half of the transition zone, reorganizes and then rapidly disassociates. As in the normal line, pairs of vascular bundles diverge into the central vein; the first pair eventually unites with the ventral-most bundle to form the central vein's large vascular column. Unlike the normal line, the vascular tissue destined for the lateral veins disperses as a partial ring of bundles around the periphery of the upper transition zone. The dorsal-most of these bundles diverge into the secondary veins of the lower leaf lobes before the outer lateral veins form. Bundle division and a second coalescence phase sort the remaining peripheral bundles into the outer and inner lateral veins. As invagination begins to separate the outer lateral veins, large peripheral bundles become the vascular columns in the inner and outer laterals. The flanking pairs of smaller bundles in the outer laterals arise from the division of both dorsal bundles and large bundles at the points of invagination. Once the outer lateral veins separate, flanking bundles in the central and inner lateral veins reconverge and form two secondary shafts of vascular tissue. These shafts reorganize and then generate pairs of flanking bundles that diverge back into the veins as invagination completes vein separation. Typically, the last pair of flanking bundles diverges into secondary veins before invagination is complete. As in normal leaves, further branching into the remaining secondaries is accompanied by the divergence of the dorsal most bundles in each vein followed by the division and divergence of the vascular columns. Vein fusion, thus, is the delay of vein formation rather then the cohesion of formed structures.

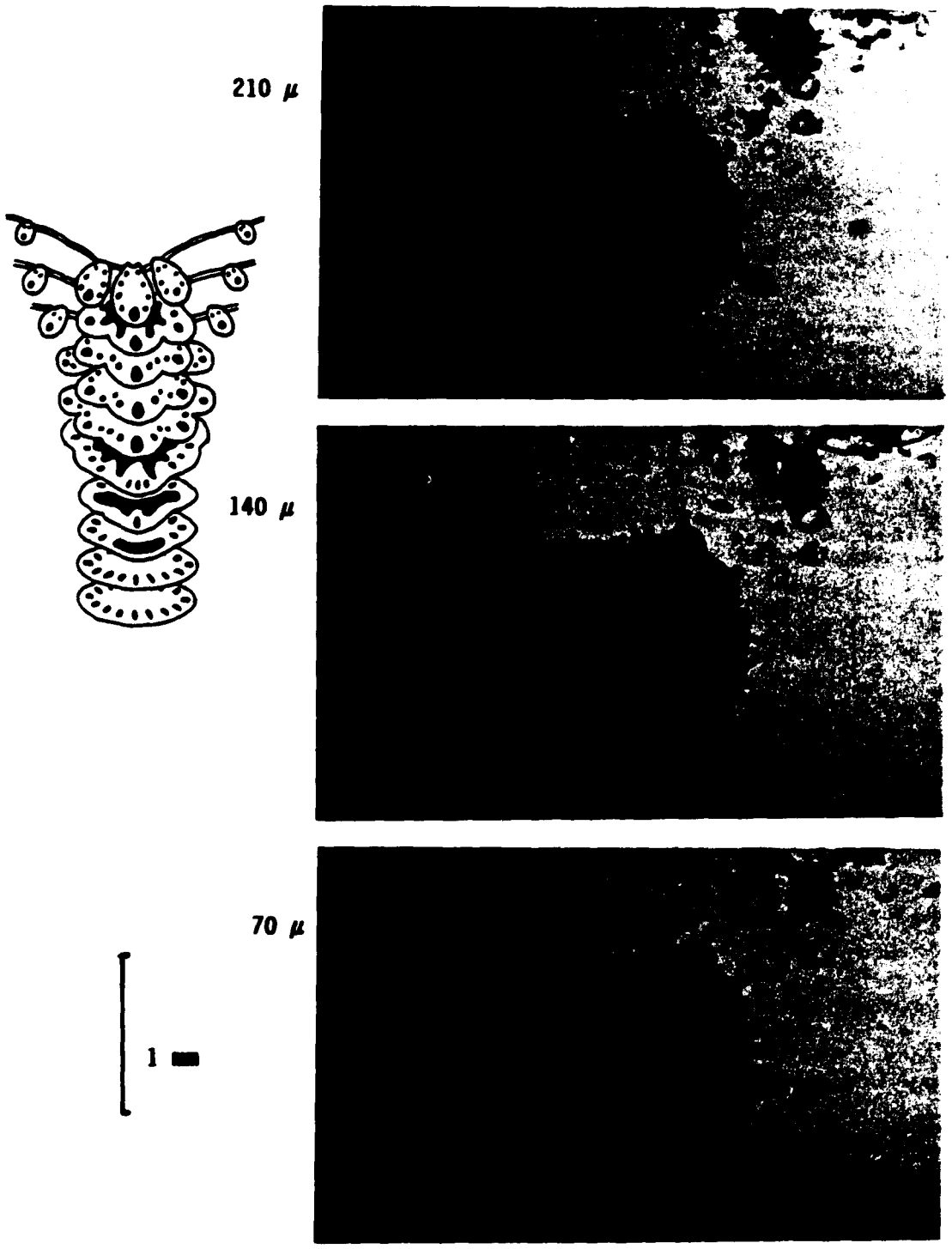


Figure 3.9. Fused vein leaf serial cross sections; 70, 140 and 210 microns from the petiole/leaf blade junction.

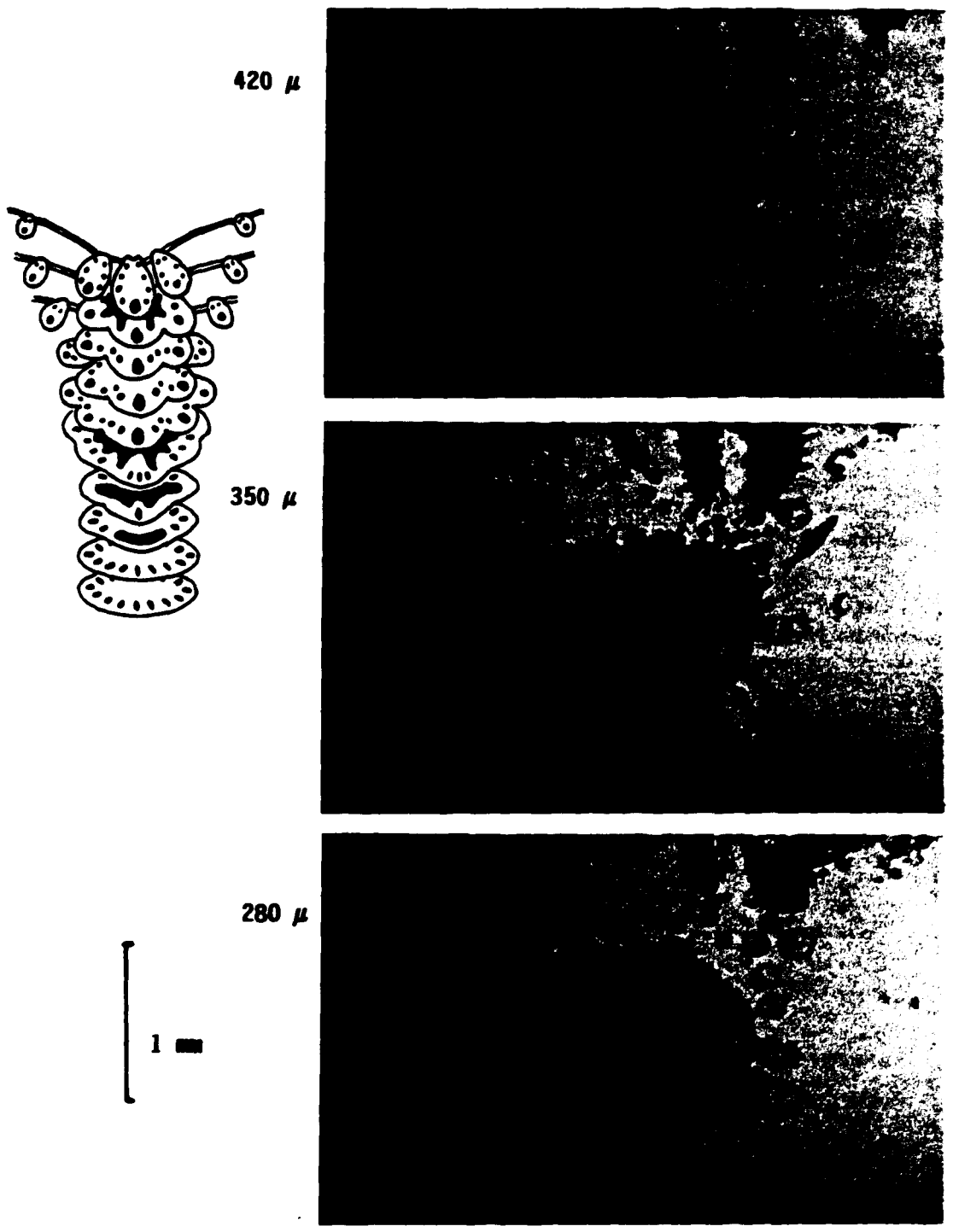


Figure 3.10. Fused vein leaf serial cross sections; 280, 350 and 420 microns from the petiole/leaf blade junction.

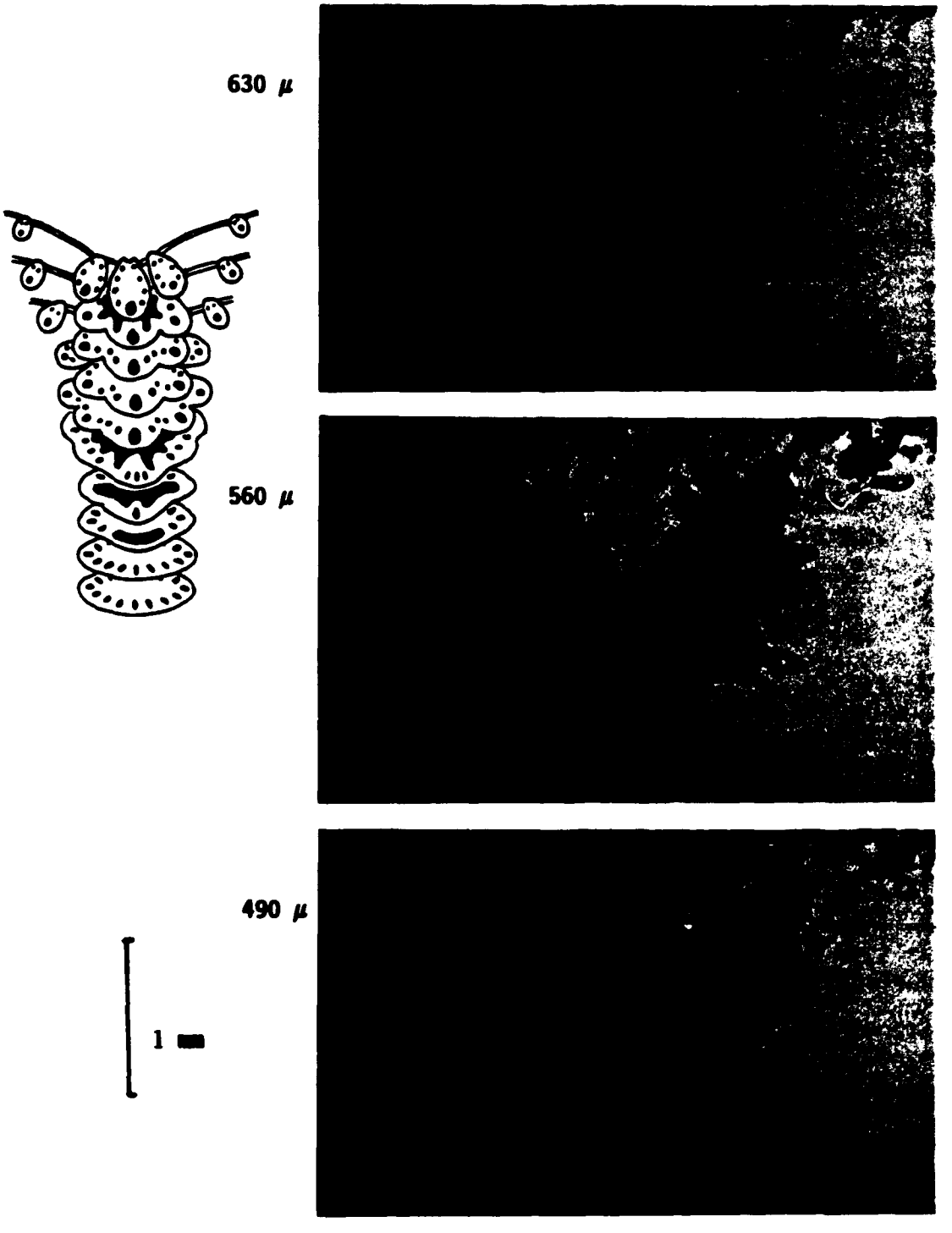


Figure 3.11. Fused vein leaf serial cross sections; 490, 560 and 630 microns from the petiole/leaf blade junction.

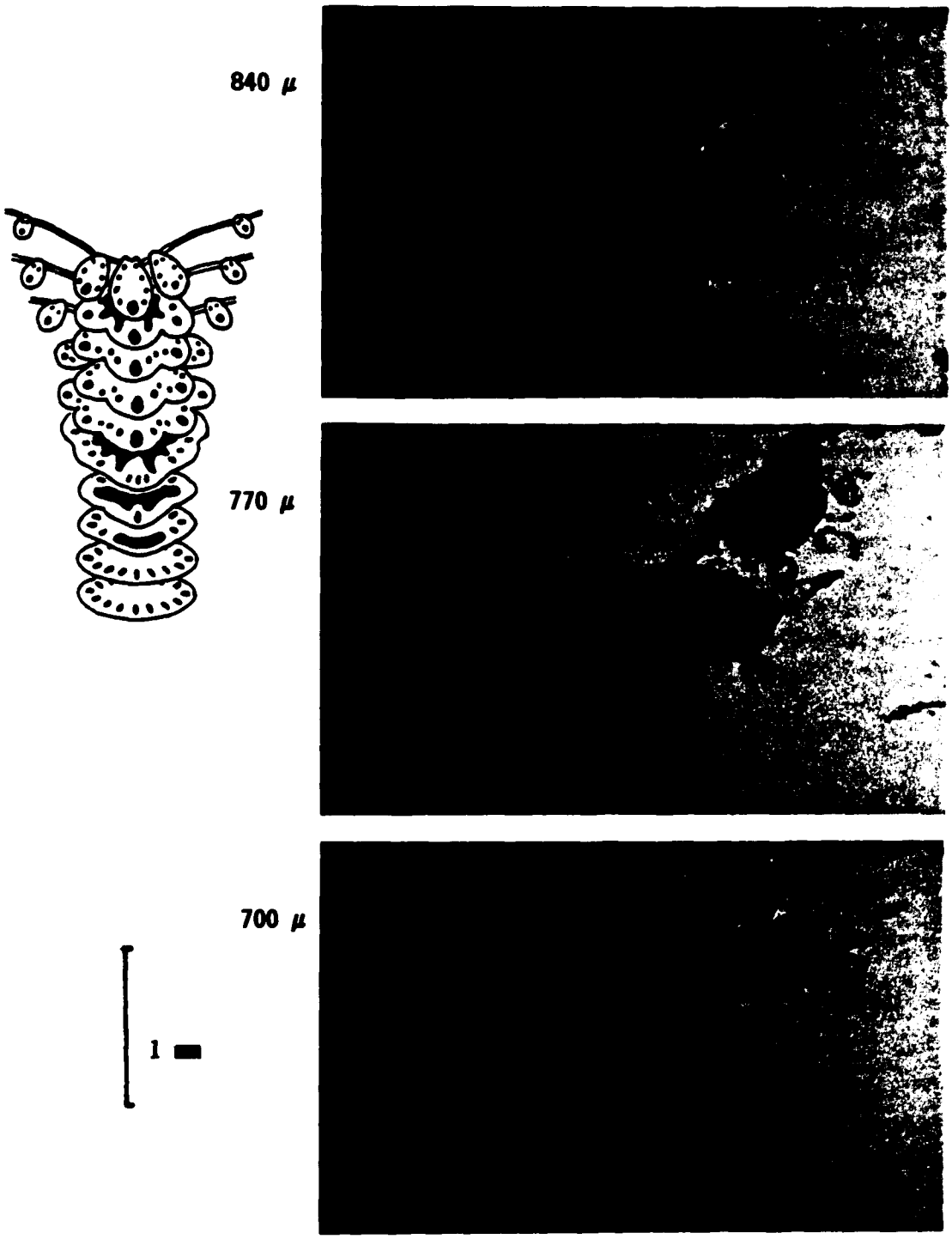


Figure 3.12. Fused vein leaf serial cross sections; 700, 770 and 840 microns from the petiole/leaf blade junction.

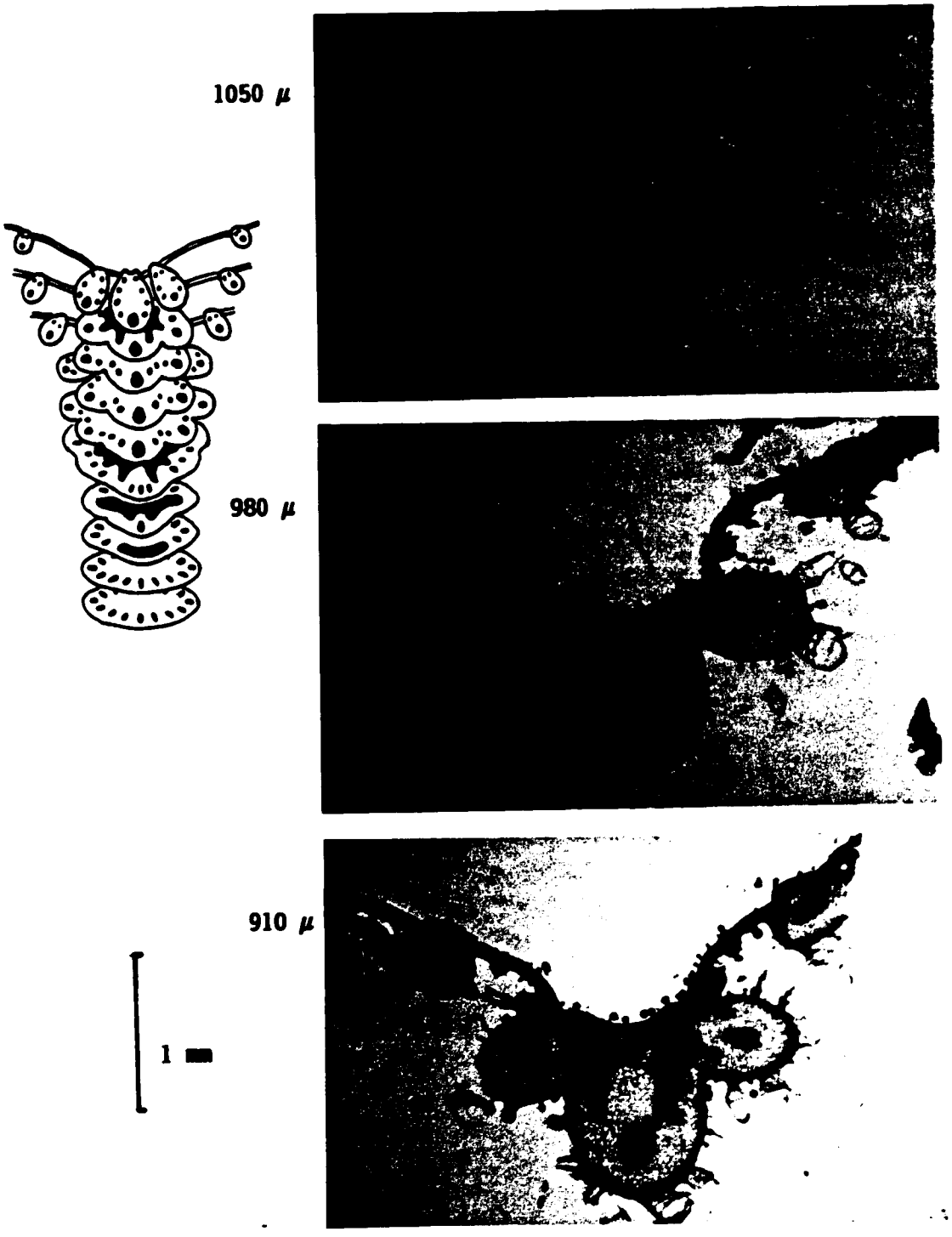


Figure 3.13. Fused vein leaf serial cross sections; 910, 980 and 1050 microns from the petiole/leaf blade junction.

Presumptive Cause of Fused Vein Structure. In typical dicot leaf development (Fahn 1990), the leaf blade results from lateral marginal meristem activity along the sides of the elongating leaf axis followed by plate meristem activity in the expanding lamina. Development of the midrib and its vascular system generally precedes lamina expansion. Differential growth of the marginal meristem creates leaf lobes, while the repression of marginal meristem activity forms petioles.

Aspects of fused vein anatomy suggest its structure results from the blurring of the petiole/leaf blade boundary through the interference in marginal meristem activity. The vascular arrangement of a fused vein leaf can be viewed as a hybrid between petiole and leaf vein. In the ventral half of the transition zone, the central vein develops more or less normally. Vascular development, dispersal and arrangement are similar to that of a normal leaf, occurring with the initial invagination of the epidermis and cortex. This pattern may result from midrib formation preceding lamina expansion. In the dorsal half of the transition zone, however, the secondary dispersal of vascular tissue forms a partial ring of bundles similar to that of the petiole. The bundles are large, equidistant and peripheral; their dispersal occurs as the coalesced shaft of vascular tissue gravitates to the dorsal half of the zone. This hybrid structure implies that marginal meristem activity is partially repressed along a dorsal ventral plane. Repression subsequently prevents the localized formation and activity of plate meristems thus causing the absence or reduction of intraveinal leaf blade. Repression is incomplete, however, because the leaf blade does develop extraveinally.

Interference of marginal meristem activity is further implicated by the similarity between leaf lobe configuration and fused vein expression. The first true leaves of *C. pepo* have three shallow lobes; as leaf number increases five lobes are produced and the leaf sinuses deepen (Whitaker and Davis, 1962). Fused vein expression has a similar pattern; expression is delayed until the fourth to tenth leaf and vein fusion increases with leaf number. In addition the greatest vein fusion was observed in fused vein segregants with the deepest leaf lobes.

An epigenetic model of plant patterning asserts that meristem activities are induced, determined and maintained through a cascade of decreasing developmental options in response to competing molecular signals (Sachs, 1991). Work with leaf mutants in *Pisum sativum* has shown that the compartmentalization of leaves into stipules, leaflets and tendrils results from homeotic-like control of the size, growth and division of leaf meristems (Marx 1987, Meicenheimer et al, 1983; Young, 1983; Gould et al 1986). Accordingly, the fused vein trait may disrupt the petiole/ leaf blade boundary in *C. pepo* by altering the distribution of a signal or signals responsible for marginal meristem activity. Instead of repression ending at presumptive petiole/leaf blade juncture, it extends into the leaf blade, where competition with signals for midrib development result in fused vein formation.

Chapter IV

Genetic Analysis of the Fused Vein Trait

Abstract

The fused vein trait in *Cucurbita pepo* L. is a potential seed production marker for hull-less seeded cultivars. It is characterized by the partial fusion of the primary leaf veins and a distinctive leaf pucker. This study investigated its expression, inheritance, and possible linkage to the hull-less seed trait. The fused vein/hull-less inbred, NH2405, was crossed to a normal/hull-less line, NH614, and a normal/hulled line, NHBP10. Reciprocal F_1, F_2, F_3, and backcross populations were generated in field and greenhouse environments and examined for leaf and seed segregation. Although the fused phenotype is stable in NH2405, it exhibited a continuum of expression in segregating populations; fusion varied from slight (1-5 cm) to extreme (10-20 cm) and the onset of fused leaf production ranged from the fourth to the tenth leaf stage. Inheritance ratios varied with population, conditions of production, and direction of cross. Most populations fit either a single or double recessive gene model, however, a quarter of the populations showed no or low fused vein recovery. A feasible explanation for the distorted inheritance is that the fused vein trait is a gametophytic subvital, governed by a single recessive gene, *fv*. Although less likely, a double recessive, subvital model cannot be ruled out. Contingency chi square analysis indicated a moderate association of the fused vein and hull-less traits. Assuming single gene inheritance and linkage, analysis of pooled F_2 and testcross data by the maximum likelihood method yielded a map distance of 29.2 $\pm$ 3 map units.

Introduction

A cultivar's purity is continually challenged by natural mutation, uncontrolled outcrossing, and accidental contamination by human error. Rouging, the removal of offtypes prior to flowering, is a routine seed production method to maintain purity and prevent subsequent contamination. Unfortunately, squash cultivars have similar juvenile morphologies and are distinguished primarily by fruit characteristics. Rouging has limited value if offtypes cannot be discerned until after flowering.

Hull-less seeded squash cultivars are particularly prone to contaminations. They are morphologically similar to many hulled pumpkins and are maintained and produced in the same agricultural region. Their seed acreage is comparatively small and adequate isolation is difficult to obtain. The hull-less trait is determined by a maternally expressed recessive gene, *n* (Stuart, 1983). Outcrossing by hulled genotypes can remain undetected for two generations allowing widespread contamination of stock and commercial seed lots.

A new leaf mutant, fused vein, has potential as a rouging marker for squash. The fused vein trait is expressed prior to flowering and imparts a distinctive, readily visible, leaf morphology. The trait is rare. Limiting its use to hull-less material would provide broad outcross detection from hulled contaminants.

The purpose of this study was to assess the genetic capacity of the fused vein trait as a production marker. Using selected breeding lines the trait's inheritance, expressivity and possible linkage to the hull-less seeded trait were determined.

Materials and Methods

Plant Material. *Cucurbita pepo* L. inbred lines, NH2405 (FL), NH614 (NL), NHBP10 (NH), and NH7210 (MFL) (Dr. J. Brent Loy, Department of Plant Biology, UNH, Durham, NH) were used to study the inheritance of the fused vein trait. NH2405, a hull-less seeded F_7 line, served as the fused vein parent in all studies. The lines NH614, a hull-less F_8, and NHBP10, a homozygous hulled F_5, functioned as normal leaf parents. NHBP10's hulled genotype allowed for the testing of linkage between the fused vein and hull-less seed traits. NH7210, a hull-less F_5, is distantly related to NH2405 (Figure. 1.1); its moderately fused phenotype enabled the examination of complementation.

Inheritance Study I. In the spring of 1990, hand pollinated reciprocal crosses were made between NH2405 and NH614 in the UNH greenhouse. The following summer, eleven F_2 populations, sixteen fused vein backcrosses and nine normal leaf backcrosses were generated over a three week period in the field at the Woodman Horticultural Farm, Durham, NH. In the fall of 1990, 48 seeds each of seven selected F_2 populations and 30 seeds each of 17 selected backcrosses were sown in six inch pots in the UNH greenhouse. Resulting plants were grown until the tenth leaf stage and scored for leaf type. In the summer of 1991, 100 seeds of each F_2 population, 50 seeds of each fused vein backcross population, and 50 seeds each of four selected normal backcross populations were planted on 31 May at the Woodman Farm. Following droughty weather and poor stand establishment, the remainder of F_2 and backcross seeds were used to overplant on 10 June. Resulting plants were scored for leaf type at the tenth leaf stage, at first flower, and

following peak bloom. During the summer of 1991, F_3 populations were generated by hand pollination from F_2 plants exhibiting various leaf phenotypes. The following fall, nine F_3 populations were grown and scored in the greenhouse in the manner used previously for the F_2 populations. Chi square analyses were performed for segregating F_2, backcross and F_3 populations, using one and two gene models for determining expected ratios. Field and greenhouse inheritance data were pooled for individual populations. F_2 and backcross population sizes and genetic models were selected based on observation of initial breeding material. Subsequently, population sizes were too small to effectively test a three gene model.

Inheritance Study II. In the spring of 1991, four F_2 and six testcross populations were generated using NH2405 x NHBP10 F_1 (supplied by J.B. Loy) and NH2405 in the UNH greenhouse. That same summer, 50 seeds from each population were planted, overplanted, and scored for leaf type as in the previous study. Seed phenotype was determined in mid September. Ripe fruit from each plant were sliced open and allowed to sit for 24 hours, which partially desiccated the exposed flesh and seeds and facilitated phenotype determination. F_3 populations were generated by hand pollination during the summer from selected F_2 plants. As in the previous study, 40 seeds each of seven F_3 populations were grown and scored in the greenhouse during the spring of 1992. Chi square analyses were performed on F_2 and testcross populations to examine segregation and independent assortment of the fused vein and hull-less seed traits. F_3 populations were analyzed only for fused vein segregation. As in the first study, one and two gene models were used to determine expected ratios. The maximum likelihood

method (Allard, 1956) was used to calculate recombination frequency and standard error .

Inheritance Study III. In the spring of 1991, three F_2 populations, three fused vein backcrosses, and three moderately fused vein backcrosses were generated using NH2405 x NHBP10 F_1 (supplied by J.B. Loy), NH2405 and NH7210 in the greenhouse by hand pollination. That same summer, 25 seeds from each population were planted, overplanted, and scored for leaf type as in the previous studies.

Results

Recessive Inheritance. Throughout primary plant growth, F_1 plants (NH2405 x NH614, NH614 x NH2405, and NH2405 x NHBP10) produced leaves of normal phenotype. Normal backcross progeny also developed normal leaves and confirmed the recessive nature of the fused vein trait (Table 4.1). Late in the 1990 and 1991 growing seasons, however, some F_1 plants derived from NH2405 and NH614 developed fused vein terminals on one or more lateral stems. In 1991, sixty-two fused vein terminals were observed among 158 F_1 plants. Rooted greenhouse cuttings of twenty-five altered terminals continued to produce fused vein leaves for four months before succumbing to pests and low light levels. Altered terminals were also noted in 1991 among normal backcross progeny derived from the same parents.

Phenotypes. F_2, F_3 and backcross plants were classified into three categories: normal, fused vein, and sectored. Progeny were considered

Table 4.1. Dominance of normal phenotype in backcross populations derived from crosses between NH2405 (fused vein) and NH614 (normal).

Cross	Normal	Fused Vein
NH614 x (NH2405 x NH614)		
90-1	48	0
90-2	29	0
90-3	30	0
(NH2405 x NH614) x NH614		
90-1	50	0
90-2	27	0
90-3	30	0
NH614 x (NH614 x NH2405)		
90-1	45	0
(NH614 x NH2405) x NH614		
90-1	51	0
90-2	28	0

normal if they generated normal leaves until the tenth leaf stage in the greenhouse and until peak bloom in the field.

Plants were designated fused vein if continual fused vein leaf production began by the tenth leaf stage. There was variation of expression, however, among the fused vein progeny. The onset of fused leaf production ranged from the fourth to the tenth leaf stage. The extent of fusion varied from slight (1-5 cm) to pronounced (10-20 cm)(Figure 4.1); the greatest vein fusion occurred in plants with the earliest onset of fused leaves and the deepest leaf lobes. The extent of fusion also increased with leaf number within each plant but stabilized by the twentieth leaf stage. Variation of expression was visibly greater between F_3 populations than within; fused vein plants within a population showed similar degrees of fusion.

Sectored plants were observed only in field grown populations derived from NH2405 and NH614. They produced both normal and fused leaves in one of two general patterns. Either fused and normal leaves alternated on a stem or different stems had different leaf phenotypes. The sectored plants comprised 5.2 percent of the field grown plants (Table 4.2) and were omitted from segregation analyses because their phenotypes were ambiguous.

F_2 and Testcross Segregation. Fused vein segregation in F_2 and testcross populations derived from NH2405 and NH614 exhibited considerable heterogeneity (Tables 4.3 and 4.4). Fused vein plants were recovered in both NH2405 and NH614 cytoplasms. Collectively, eleven populations fit ratios expected for a single recessive gene (F_2: 3 normal to 1 fused, testcross: 1 normal to 1 fused) at a confidence level of .05 or greater.

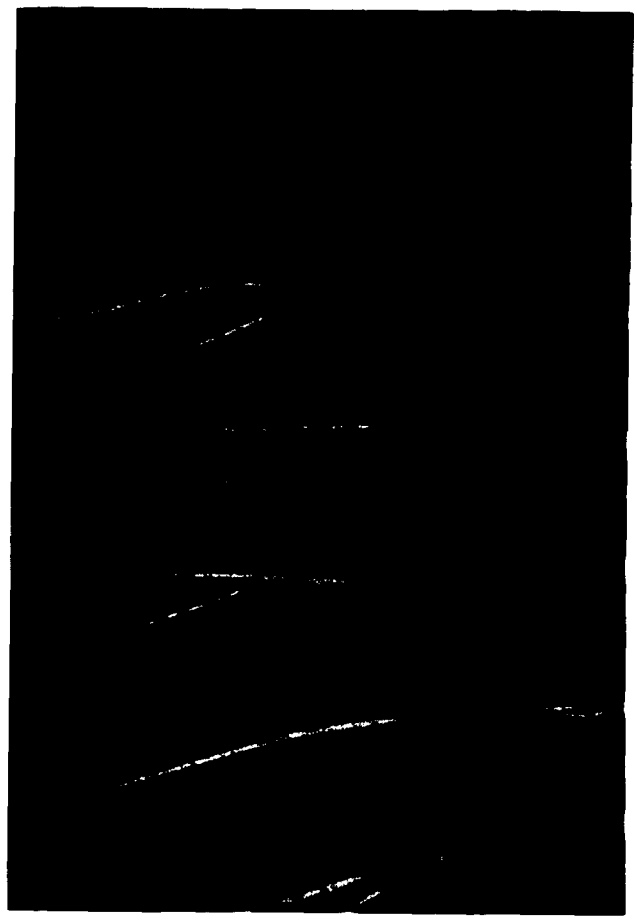


Figure 4.1. Fused vein phenotypes for F2 and backcross populations of *Cucurbita pepo*.

Table 4.2. Percent sectored progeny for field grown F_2 and testcross populations derived from crosses between NH2405 (fused vein) and NH614 (normal).

Cross	Normal	Fused Vein	Sectored	% Sectored
NH2405 x NH614 F_2				
90-1	45	15	3	4.8
90-2	34	2	2	5.3
90-3	48	0	1	2.0
90-4	42	4	2	4.2
90-5	25	9	2	5.6
90-6	66	0	0	0.0
90-7	40	22	4	6.1
90-8	53	20	4	5.2
NH614 x NH2405 F_2				
90-1	78	2	6	7.0
90-2	88	0	0	0.0
90-3	72	0	1	1.4
NH2405 x (NH2405 x NH614)				
90-1	18	13	4	11.4
90-2	6	6	1	7.7
90-3	20	9	5	14.7
90-4	20	14	3	8.1
90-5	28	2	1	3.2
(NH2405 x NH614) x NH2405				
90-1	6	33	3	7.1
90-2	12	2	1	6.7
90-3	10	17	1	3.6
90-4	30	5	10	22.2
90-5	14	9	1	4.2
NH2405 x (NH614 x NH2405)				
90-1	12	4	0	0.0
90-2	05	7	1	7.7
90-3	13	7	1	4.8
90-4	12	7	2	9.1
(NH614 x NH2405) x NH2405				
90-1	11	7	0	0.0
90-2	16	30	0	0.0
Totals	825	246	59	5.2

Eight populations fit a two gene double recessive model (F_2: 15 normal to 1 fused, testcross: 3 normal to 1 fused). Eight populations had either no fused vein plants or insufficient plants to fit either model. One testcross population, having 75 percent fused vein plants, exceeded the expected recovery for a recessive trait. Although reciprocal testcrosses showed variable fused vein recovery, higher recovery was obtained more often with the fused vein parent as the pollen source. In contrast, segregating populations derived from NH2405 and NHBP10 were comparatively homogeneous (Tables 4.5 and 4.6). All four F_2 and five of six testcross populations fit ratios for a double recessive model with one F_2 also fitting a monohybrid ratio.

Table 4.3. Fused vein segregation for F_2 populations[a] derived from crosses between NH2405 (fused vein) and NH614 (normal).

Cross	Phenotype[b]			X^2[d]	P	X^2[d]	P
	N	F	S[c]	3:1		15:1	
NH2405 x NH614 F_2							
90-1	86	22	3	1.23	.50-.25	36.75	<.005
90-2	80	4	2	18.35	<.005	0.32	.75-.50
90-3	96	0	1	32.00	<.005	6.40	.02-.01
90-4	87	7	2	15.45	<.005	0.23	.75-.50
90-5	25	9	2	0.04	.90-.75	23.73	<.005
90-6	66	0	0	22.00	<.005	4.40	.05-.02
90-7	40	22	4	3.63	.10-.05	90.43	<.005
90-8	53	20	4	0.22	.75-.50	55.72	<.005
NH614 x NH2405 F_2							
90-1	126	2	6	37.50	<.005	4.80	.05-.02
90-2	136	0	0	45.33	<.005	9.07	<.005
90-3	120	0	1	40.00	<.005	8.00	<.005
Totals	915	86	25	215.79	<.005	239.85	<.005
Pooled X^2				143.74	<.005	9.37	<.005
Homogeneity X^2				72.05	<.005	230.48	<.005

[a] Field and greenhouse plantings combined.
[b] Phenotypes: N normal, F fused vein, S sectored plant.
[c] Sectored data omitted from analysis.
[d] X^2 df: individual, 1; total, 11; pooled, 1; homogeneity, 10.

Table 4.4. Fused vein segregation for testcross populations[a] derived from crosses between NH2405 (fused vein) and NH614 (normal).

Cross	Phenotype[b] N	F	S[c]	X^{2d} 1:1	P	X^{2d} 3:1	P
NH2405 x (NH2405 x NH614)							
90-1	34	27	4	0.80	.50-.25	12.07	<.005
90-2	29	13	1	6.10	.02-.01	0.79	.50-.25
90-3	46	13	5	18.46	<.005	0.28	.75-.50
90-4	36	28	3	1.00	.50-.25	12.00	<.005
90-5	54	6	1	38.40	<.005	7.20	.01-.005
(NH2405 x NH614) x NH2405							
90-1	17	52	3	17.75	<.005	93.34	<.005
90-2	41	3	1	32.82	<.005	7.76	.01-.005
90-3	27	27	1	0.00	>.999	18.00	<.005
90-4	56	7	10	38.11	>.005	6.48	.02-.01
90-5	15	9	1	1.50	.25-.10	2.00	.25-.10
NH2405 x (NH614 x NH2405)							
90-1	31	13	0	7.36	.01-.005	0.48	.50-.25
90-2	20	22	1	0.10	.90-.75	16.79	<.005
90-3	32	18	1	3.92	.05-.02	3.23	.10-.05
90-4	32	17	2	4.59	.05-.02	2.46	.25-.10
(NH614 x NH2405) x NH2405							
90-1	28	20	0	1.33	.25-.10	7.11	.01-.005
90-2	33	43	0	1.32	.25-.10	40.42	<.005
Totals	531	318	34	173.56	<.005	230.41	<.005
Pooled X^2				53.44	<.005	70.25	<.005
Homogeneity X^2				120.12	<.005	160.16	<.005

[a] Field and greenhouse plantings combined
[b] Phenotypes: N normal, F fused vein, S sectored plant.
[c] Sectored data omitted from analysis.
[d] X^2 df: individual, 1; total, 11; pooled, 1; homogeneity, 10.

Table 4.5. Fused vein segregation for F_2 populations derived from crosses between NH2405 (fused vein) and NHBP10 (normal).

Cross	Phenotype[a] N	F	X^{2b} 3:1	P	X^{2b} 15:1	P
NH2405 x NHBP10 F_2						
91-1	24	4	1.71	.25-.10	3.09	.10-.05
91-2	27	0	9.00	<.005	1.80	.25-.10
91-3	36	0	12.00	<.005	2.40	.25-.10
91-S	44	1	12.45	<.005	1.25	.50-.25
Total	131	5	35.16	<.005	8.54	<.005
Pooled X^2			32.98	<.005	1.54	.25-.10
Homogeneity X^2			2.18	.75-.50	7.00	.10-.05

[a] Phenotypes: N, normal; F, fused vein.
[b] X^2 df: individual, 1; total, 4; pooled, 1; homogeneity, 3.

Table 4.6. Fused vein segregation for testcross populations derived from crosses between NH2405 (fused vein) and NHBP10 (normal).

Cross	Phenotype[a] N	F	X^2[b] 1:1	P	X^2[b] 3:1	P
NH2405(NHBP10 x NH2405)						
91-1	24	10	5.76	.02-.01	0.35	.75-.50
91-2	21	3	13.50	<.005	2.00	.25-.10
91-3	9	1	6.40	.02-.01	1.20	.50-.25
91-4	8	2	3.60	.10-.05	0.13	.75-.50
91-5	8	2	3.60	.10-.05	0.13	.75-.50
(NH2405 x NHBP10)NH2405						
91-1	43	2	37.36	<.005	10.14	<.005
Total	113	20	70.22	<.005	13.95	.05-.02
Pooled X^2			65.03	<.005	7.04	.01-.005
Homogeneity X^2			5.19	.50-.25	6.91	.25-.10

[a] Phenotypes: N, normal; F, fused vein.
[b] X^2 df: individual, 1; total, 6; pooled, 1; homogeneity, 5.

F_3 Segregation. Inheritance data for F_3 populations descending from crosses between NH2405, NH614 and NHBP10 are provided in Table 4.7. As expected for a recessive trait, F_3 populations generated from fused vein F_2 plants contained only fused vein individuals. Of the ten F_3 populations produced from normal F_2 plants, two segregated as a single recessive gene and eight contained all normal plants. The segregating F_3s were derived from F_2 populations with high fused vein frequencies while the nonsegregating F_3s were derived from F_2 populations containing few or no fused vein plants. The eight NH2405 x NHBP10 F_3 populations were derived from F_2 populations that fit a double recessive model, however, one out of eight segregating populations is fewer than expected for either a double (8 segregating to 7 all normal) or single recessive model (2 segregating to 1 all normal).

The two F_3 populations derived from sectored F_2 progeny came from separate plants. Both plants had a normal main stem, one fused vein

lateral stem and one normal lateral stem. *C. pepo* 's alternating pattern of bloom necessitated pollination of female lateral stem flowers by main stem male flowers. The F_3 obtained from a fused vein lateral segregated in a 3:1 ratio, suggesting a heterozygous genotype for the sectored plant. In contrast, progeny from a normal stem of second sectored F_2 were all normal indicating a homozygous dominant genotype.

Table 4.7. Fused vein segregation for F_3 populations derived from crosses between NH2405 (fused vein), NH614 (normal) and NHBP10 (normal).

Cross	F_2 Phenotype	F_2 FVFQ[a]	Phenotype[b] N	F	X^{2c} 3:1	P	X^{2c} 15:1	P
NH2405 x NH614 F_3								
91-13	Fused	0.20	0	44				
91-15	Fused	0.20	0	47				
91-16	Fused	0.20	0	54				
91-14	Normal	0.20	32	13	0.40	.75-.50	39.36	<.005
91-33	Normal	0.00	43	0				
91-41	Sectored[d]	0.07	33	13	0.36	.75-.50	38.04	<.005
91-71	Sectored[e]	0.35	47	0				
NH2405 x NHBP10 F_3								
91-11	Normal	0.14	29	13	0.79	.50-.25	43.74	<.005
91-21	Normal	0.00	42	0				
91-22	Normal	0.00	42	0				
91-31	Normal	0.00	37	0				
91-32	Normal	0.00	42	0				
91-S1	Normal	0.02	15	0				
91-S2	Normal	0.02	43	0				
91-S3	Normal	0.02	40	0				

[a] FVFQ: fused vein frequency in F2 population
[b] Phenotypes: N normal, F fused vein.
[c] X^2 degrees of freedom = 1
[d] Fruit obtained from fused vein stem.
[e] Fruit obtained from normal stem.

Hull-less Seed Inheritance. Observed segregation for seed type in populations derived from crosses between NH2405 and NHBP10 did not conform to single gene inheritance as reported in the literature (Tables 4.8 and 4.9). A majority of the F_2 and testcross populations agreed with both

single and double recessive models. Pooled F_2 data failed to fit either model (one gene: $X^2_{3:1}$ = 7.69; P = .01-.005 or two genes: $X^2_{15:1}$ = 16.60; P <.005); while pooled testcross data supported double recessive inheritance ($X^2_{3:1}$ = 2.41; P = .25-.10). Although recovery of hull-less seed progeny was less than expected, it exceeded the recovery of fused vein progeny in each of the populations (Tables 4.5 and 4.6)

Table 4.8. Hull-less seed segregation for F_2 populations derived from crosses between NH2405 (hull-less) and NHBP10 (hulled).

Cross	Phenotype[a] H	L	X^2[b] 3:1	P	X^2[b] 15:1	P
NH2405 x NHBP10 F_2						
91-1	24	4	1.71	.25-.10	3.09	.10-.05
91-2	24	3	2.78	.10-.05	1.09	.50-.25
91-3	33	3	5.33	.02-.01	0.27	.75-.50
91-S	35	10	0.19	.90-.75	19.59	<.005
Total	106	20	10.01	.05-.02	24.04	<.005
Pooled X^2			7.69	.01-.005	16.60	<.005
Homogeneity X^2			2.32	.75-.50	7.44	.10-.05

[a] Phenotypes: H, hulled; L, hull-less.
[b] X^2 df: individual, 1; total, 4; pooled, 1; homogeneity, 3.

Table 4.9. Hull-less seed segregation for testcross populations derived from crosses between NH2405 (hull-less) and NHBP10 (hulled).

Cross	Phenotype[a] H	L	X^2[b] 1:1	P	X^2[b] 3:1	P
NH2405(NHBP10 x NH2405)						
91-1	28	6	14.24	.25-.10	0.98	.50-.25
91-2	16	8	2.67	.10-.05	0.92	.50-.25
91-3	8	2	3.60	.02-.01	0.13	.75-.50
91-4	7	3	1.60	.75-.50	0.13	.75-.50
91-5	6	4	0.40	.90-.75	1.20	.50-.25
(NH2405 x NHBP10)NH2405						
91-1	27	18	1.80	.25-.10	5.40	.02-.01
Total	92	41	24.31	<.005	8.76	.25-.10
Pooled X^2			19.56	<.005	2.41	.25-.10
Homogeneity X^2			4.75	.50-.25	6.35	.50-.25

[a] Phenotypes: H, hulled; L, hull-less.
[b] X^2 df: individual, 1; total, 6; pooled, 1; homogeneity, 5.

Linkage of the Fused Vein and Hull-less Traits. Combined segregation of leaf and seed types for populations derived from NH2405 and NHBP10 are presented in Table 4.10. Poor stand establishment resulted in several small populations which obstructed analysis of individual populations. Segregation patterns across populations, however, were sufficiently homogeneous and permitted the pooling of data.

There was significant lack of fit of the pooled F_2 data to ratios expected by the independent assortment and normal segregation of one hull-less gene with either one ($X^2_{9:3:3:1}$ = 44.44; P <.005) or two ($X^2_{45:15:3:1}$ = 10.99; P =.02-.01) fused vein genes. Pooled testcross data also failed to fit ratios predicted by the same models ($X^2_{1:1:1:1}$ = 104.14; P <.005 and $X^2_{3:3:1:1}$ = 34.27; P <.005). Failure of the data to match the expected ratios and the skewed hull-less seed inheritance suggested an association between the hull-less gene to the fused vein trait, possibly due to linkage.

Since both F_2 and testcross data were homogeneous, contingency tests for independent assortment were performed using the pooled data (Table 4.11). Test of the F_2 indicated independent assortment (X2 = 2.63; P =.25-.10), while testcross results supported association of the two traits (X^2 = 4.05; P =.05-.02). Assuming a single gene for the fused vein trait, combined analysis of pooled F_2 and testcross data by the maximum likelihood method (Allard, 1956) yielded a recombination value of 29.2 $\pm$ 3.0 percent. This moderate level of recombination, the skewed hull-less seed inheritance, and the positive testcross contingency test support an hypothesis of linkage between fused vein and hull-less seed genes.

Table 4.10. Independent assortment of fused vein and hull-less traits in F_2 and testcross populations derived from crosses between NH2405 (fused vein, hull-less) and NHBP10 (normal, hulled).

Cross	Phenotype[a]				Chi Square NH:NL:FH:FL			
	NH	NL	FH	FL				
F_2s					X^2[b] 9:3:3:1	P	X^2[b] 45:15:3:1	P
NH2405 x NHBP10								
91-1	21	3	3	1	4.00	.50-.25	4.91	.25-.10
91-2	24	3	0	0	12.70	.01-.005	4.76	.25-.10
91-3	33	3	0	0	19.11	<.005	8.09	.05-.02
91-S	35	9	0	1	13.35	<.005	2.82	.50-.25
Total	113	18	3	2	49.16	<.005	20.58	.10-.05
Pooled X^2					44.44	<.005	10.99	.02-.01
Homogeneity X^2					4.72	.90-.75	9.59	.50-.25
Testcrosses					X^2[c] 1:1:1:1	P	X^2[c] 3:3:1:1	P
NH2405(NHBP10 x NH2405)								
91-1	23	1	5	5	34.24	<.005	19.33	<.005
91-2	13	8	3	0	16.33	<.005	4.89	.25-.10
91-3	8	1	0	1	16.40	<.005	8.13	.05-.02
91-4	6	2	1	1	6.80	.10-.05	2.27	.75-.50
91-5	6	2	0	2	7.60	.10-.05	3.87	.50-.25
(NH2405 x NHBP10)NH2405								
91-1	26	17	1	1	40.96	<.005	12.54	.01-.005
Total	82	31	10	10	122.33	<.005	51.03	<.005
Pooled X^2					104.14	<.005	34.27	<.005
Homogeneity X^2					18.19	.50-.25	16.76	.50-.25

[a] Phenotypes: NH, normal/hulled; NL, normal/hull-less; FH, fused vein/hulled; FL, fused vein/hull-less.
[b] X^2 df: individual, 3; total, 12; pooled, 3; homogeneity, 9.
[c] X^2 df: individual 3; total, 18; pooled, 3; homogeneity, 15.

Table 4.11. Chi- square contingency tests for the independent assortment of the fused vein and hull-less seed traits in populations derived from NH2405 (fused vein, hull-less) and NHBP10 (normal, hulled).

Pooled F_2

	Hulled	Hull-less		
Normal	113 (111.72)[a]	18 (19.25)	131	$X^2 = 2.63$
Fused	3 (4.29)	2 (0.74)	5	df = 1
	116	20	136	.25 > P > .10

Pooled Testcross

	Hulled	Hull-less		
Normal	82 (78.17)	31 (34.83)	113	$X^2 = 4.05$
Fused	10 (13.83)	10 (6.17)	20	df = 1
	92	41	133	.05 > P > .02

[a] Values in parenthesis are the expected values for each class. They are the product of the total population times the individual probabilities for two phenotypes within each class. F_2 probabilities: hulled, 0.853; hull-less, 0.147; normal, 0.963; fused, 0.037. Testcross probabilities: hulled, 0.692; hull-less, 0.308; normal, 0.850; fused, 0.150.

Complementation. All F_1, F_2 and backcross plants derived from crosses between the fused vein, NH2405, and moderately fused vein, NH7210, lines produced fused leaves beginning with the fourth to sixth leaf stage and then throughout vegetative growth. As with the normal line crosses, F_2 and backcross populations displayed a continuum of fused vein expression that varied both within and between plants. The variation, however, was subtle; the degree of fusion was no less or no greater than either parent. The observations suggested that NH2405 and NH7210 have identical fused vein genotypes, but that phenotypic expression was modified by different genetic backgrounds.

Discussion

The results do not conform to a simple genetic model based solely on the normal segregation and transmission of one or two recessive genes. Fused vein recovery varied extensively among both F_2 and testcross populations with six populations having no fused vein plants at all. Although most populations fit either a single or double recessive gene model, a quarter of the populations had insufficient fused vein plants for either model. In addition, the number of segregating NH2405 x NHBP10 F_3 populations was less than expected for a one or two gene model. The erratic and often low recovery of the fused vein trait suggest that the transmission of fused vein alleles is variously impeded during reproduction. A plausible hypothesis for fused vein inheritance is that the trait is a gametophytic subvital governed by a single recessive gene, designated *fv*. It assumes that normal gametophytes have a selective advantage during the reproductive processes which can be either accentuated or minimized by environmental conditions.

Gametophytic selection is believed to be pervasive in angiosperms (Mulcahy, 1975; Zamir, 1983). Preferential allele transmission has been demonstrated for both single and multigenic traits in several species (Keder *et al.*, 1967; Rabinowitch *et al.*, 1978; McGee and Baggett, 1992a and 1992; Ottaviano *et al.*, 1983 and 1988; Mulcahy and Mulcahy, 1975; Mulcahy *et al.*, 1975). Various environmental stresses have also been shown to cause differential allele transmission. (Zamir *et al.*, 1981,1982, and 1987; Rodriquez-Garay and Barrow, 1988; Sacher *et al.*, 1983; Mulinix and Iezzoni, 1988). Moreover, *C. pepo* pollen is highly sensitive to

environmental stress (Gay *et al.*, 1987). Both fruit and seed set in the cultivated Cucurbitaceae are adversely affected by high and low temperature, low humidity, low light intensity and poor pollen load (Porter, 1933; Mann and Robinson, 1950; Matlop and Kelly, 1973; Sedgley and Buttrose, 1978; Iapichino and Loy, 1987, Windsor *et al.*, 1987; Maestro and Alvarez, 1988; Hutton, 1988).

The segregating populations of the first inheritance study were generated from parents exposed to a fluctuating field environment throughout growth and pollination. Although plants were healthy and vigorous, they experienced daily changes in temperature, humidity and water supply. Variable stress during the three weeks of pollination would be expected to create different levels of gametic competition in each flower. Consequently, populations with different genetic distributions would be produced, accounting for the first study's heterogeneity and often low fused vein recovery. In contrast, populations in the second study were created in a relatively stable, albeit suboptimal, greenhouse environment. Moderate but constant stress from short days, pot culture, and white flies would be expected to produce populations with similar genetic distributions but with fewer then expected fused vein plants.

The F_3 data are also explained by gametophytic selection. Preferential transmission of normal alleles would have lowered the number of F_2 heterozygotes and consequently the number of segregating F_3 populations. Furthermore, the decrease of heterozygotes would have paralleled the decrease of homozygote recessives within each F_2 population. As expected, segregating F_3s were derived from F_2 populations with relatively high fused

vein frequencies.

Gametophytic selection also provides an explanation for the skewed inheritance of the hull-less seeded character. In the second inheritance study, the fused vein and hull-less traits were correlated in coupling phase. Assuming linkage, selection against fused vein alleles was also selection against hull-less alleles but at a strength inversely proportion to the map distance between the genes. The suboptimal greenhouse environment lowered the recovery of hull-less seeded progeny but not as much as fused vein recovery, because recombination events produced superior normal gametes carrying the hull-less allele.

In the first study, low fused vein recovery among both reciprocal testcrosses indicates that selection operates on both male and female gametes and may extend to the developing zygotes. Selective fruit abortion in *C. pepo* has been associated with poor pollen load and seed set (Stephenson and Windsor, 1986; and Windsor *et al.*, 1987). *C. pepo* fruit often contain a number of undeveloped seed. Although seed homozygous for fused vein appear normal and viable, selective abortion of zygotes may also contribute to the reduction of fused vein progeny. In general, however, the lowest fused vein recoveries occurred when fused and normal pollen were in competition; the highest recoveries resulted when the fused vein parent was the pollen source. This implies that for the fused vein trait, the male gamete is relatively more prone to selection than the female gamete or developing zygote.

The lack of populations fitting a single gene model in the second inheritance study, as compared to the first, challenges the validity of

a single subvital gene model for the fused vein trait. Instead, it implies a double homozygous recessive model, e.g. *ff vv*, with gametophytic selection for one or both genes. The appearance of one and two gene segregation and "no fit" populations in the first inheritance study would result from NH614 being heterozygous for one of the two presumptive genes, *Ff VV* or *FF Vv*. In the second study, NHBP10 being homozygous dominant for both genes, *FF VV*, would produce only two gene segregation or "no fit" populations in crosses with a double recessive fused vein parent, ffvv. Although possible, this model is unlikely. NH614 and NHBP10 were highly uniform F_6 and F_5 lines, respectively, when the F_1s were generated for the inheritance studies. Theoretically, 98.4 percent of NH614 genes were homozygous. Additional inheritance data from the Pollen Competition Study (following chapter) also favor a single gene model. The double gene model, however, can not be eliminated; the recessive hull-less seed gene, for example, has once remained hidden as a heterozygote until the F_6 generation in Dr. Loy's snack seed breeding program.

The variation of fused vein expression in segregating populations can be presumed to have a genetic origin. This is inferred from the distinct and stable phenotypes of NH2405 and NH7210, from the greater variation seen between F_2 populations than within, and from the uniformity of expression in F_3 populations. The rapid stabilization of expression in the F_3 suggests that relatively few genes modify expression. A genetic basis for fused vein variation implies that the trait is completely penetrant. The low recovery of fused vein individuals was not likely the result of fused vein genotypes failing to manifest the fused vein

phenotype. Incomplete penetrance would be expected to produce sister populations with similar phenotypic classes and distributions in the same environment. This is contrary to the observed results.

The appearance of fused vein terminals in F_1 plants was an unexpected and unusual manifestation of the fused vein phenotype. The continued production of fused leaves by rooted cuttings of these terminals suggested that a permanent change of genotype may have occurred. Unfortunately, attempted selfs of the subsequent plants aborted and this belief was not verified. Nevertheless, an association of this phenomenon with the mix of phenotypes found in sectored F_2 and testcross progeny can be conceived. If genotypic change generated the F_1 metamorphosis, then perhaps it also formed the sectored plants, making them genetic chimeras.

This supposition is consistent with the segregation patterns observed in the two F_3 populations derived from different sectored F_2 plants. Both sectored plants consisted of a normal main stem with two primary lateral stems, one normal and the other fused vein. *C. pepo* 's alternating pattern of bloom required lateral stem pollinations to be preformed with male flowers from the main stem. The F_3 obtained from the fruit of a fused vein lateral presumably resulted from a cross between a homozygous recessive female flower and a heterozygous male flower. As observed, it would be expected to segregate for the fused vein trait. The F_3 obtain from the fruit of a normal lateral resulted from either a cross between heterozygote and homozygous dominant flowers or between two homozygous dominant flowers. As observed, it would not be expected to segregate.

An underlying mechanism for these localized genotypic changes was not determined; however, several observations suggest further avenues of investigation. The frequent occurrence of the unexpected phenotypes opposes reoccurring mutation, gene conversion, and somatic crossovers as agents of genotypic change. A small chromosomal aberration could promote somatic segregation in heterozygotes through complete or partial chromosome loss or through mitotic nondisjunction. Consistent with the trait's gametic subvitality, chromosomal aberrations have been associated with reduced fertility (Swanson et al., 1981). However, fused by normal F_1s showed a significant increase in seed yield rather than a characteristic reduction in fertility (see following chapter).

Alternatively, transposible elements can also cause localized changes in phenotype. Two elements may be affecting fused vein expression in a manner similar to the way that *Ds* and *Ac* elements affect kernel color in *Zea mays* (Shapiro, 1983; Doring and Starlinger, 1986; Frederoff, 1989). Insertion of a transposible element may have generated the initial fused vein allele. The stability of NH2405 and NH7210 requires that the insert would be a nonautonomous element incapable of self transposition and that both lines would also be free of autonomous activator elements. Instability would be renewed when a fused vein line is crossed to a normal line carrying an activator element. In the heterozygote, copy and transposition of the nonautonomous element would be possible with each cell division; transposition into the normal allele would result in loss of dominant gene expression. In contrast, heterozygotes made with normal lines without activator elements would be incapable of transposition,

explaining the lack of phenotypic metamorphosis in crosses made with NHBP10. Contrary to observation, this model predicts that some fused vein plants in segregating populations should show reversions to the normal phenotype. It also does not explain why phenotypic metamorphosis was limited to late season terminals in F_1 plants, and why fused vein terminals were not found among normal F_2s.

The fused vein trait fulfills the genetic requirements for a rouging marker but it also has inherent weaknesses. As a rare single recessive it can provide broad outcross detection and should be easily manipulated in breeding programs. Gametic subvitality, however, will hamper its rapid transfer to desired lines. Control of pollen competition and consideration of direction of cross will be required during cultivar development. The linkage of the fused vein trait to the hull-less seeded gene, *n*, favors restricting its use to this group. However, gametic subvitality may have a detrimental affect on fertility and seed yield and must be evaluated. Finally, the variable expressivity of the fused vein trait will require selection of forms that are sufficiently fused to permit easy field identification; this may not be possible in all genetic backgrounds.

CHAPTER V

Gametophytic Selection and the Fused Vein Trait

Abstract

Four experiments were conducted to test and delineate the gametophytic subvitality of the fused vein trait in *Cucurbita pepo* L.

Gametophytic subvitality was verified by comparing pollen tube growth for fused vein and normal pollen *in situ*. Microscopic examination of partitioned, co-pollinated pistillate flowers revealed inferior fused vein gametophyte performance. Normal pollen tubes grew faster and were significantly more abundant in the style.

To further define the gametophytic selection mechanism in *C. pepo*, ovule and seed distributions were compared in open pollinated fruit of NH2405. Ovule counts of pistillate flowers showed an even distribution of ovules within the ovary. Seed counts of mature fruits, however, showed a three fold increase in seed set for the proximal half of the seed cavity. Female resource allocation favored seed development from ovules fertilized by superior male gametophytes, those with the most vigorous tube growth.

The consequences of gametophytic subvitality on seed yield and inheritance were shown by manipulating the severity of reproductive competition. Fused vein, normal and F_1 lines were pollinated with fused vein, normal, F_1, and a 50:50 pollen mix at three different pollen loads. Analysis showed that fused vein pollen generated significantly fewer seed

per fruit in all female genotypes. As a constituent in F_1 or mixed pollen, it also produced significant yield reductions at the low pollen load. In the subsequent F_2 and testcross populations, a reduction in pollen load and therefore pollen competition significantly increased the number of fused vein individuals in segregating populations.

Leaf number and area for fused vein, normal, F_1, F_2, and backcross plants were assessed for a six week period in order to test for detrimental effects on plant growth common to gametophytic subvitals. Although fused vein and normal lines differed in leaf number and size, their total leaf areas were not significantly different. F_2 and testcross plants also showed no significant difference between fused vein and normal individuals; both leaf size and number were independant of leaf morphology.

Introduction

Hull-less seeded cultivars of *Cucurbita pepo* L. are prone to repeated contamination during stock and commercial seed production (Matheson, 1991). Hull-less cultivars are morphologically similar to hulled pumpkins and are maintained and produced in the same agricultural regions. Their total acreage, however, is comparatively small and adequate isolation is difficult to obtain. The hull-less trait is governed by a maternally expressed recessive gene (Stuart, 1983). A chance outcross by a hulled cultivar can remain undetected until the following generation's seed harvest, by which time widespread contamination can occur.

Use of the fused vein trait as a rouging marker offers a possible solution. The trait is expressed prior to flowering, and imparts a distinctive, readily visible, leaf morphology. It is rare and likely governed by a single recessive gene. Combined, these attributes allow early and easy outcross detection from a broad range of potential contaminants. Inheritance studies, however, suggest that the fused vein trait is a gametophytic subvital which may limit its usefulness. Gametophytic inferiority has been associated with small plant size, reduced vigor, low fertility, and poor seed quality; all of which are important components for a seed crop (Mulcahy, 1974; Mulcahy and Mulcahy, 1975; Mulcahy *et al.*, 1975; Ottaviano *et al.*, 1980, 1983, 1988; Windsor *et al*, 1987). The purposes of the following studies were to confirm the fused vein trait's gametophytic subvitality and to determine its impact on plant growth and seed production.

Materials and Methods

In situ Pollen Tube Growth Study

Plant Material. The fused vein inbred line NH2405, the normal inbred line NH614 (J. B. Loy, Department Plant Biology, UNH, Durham, NH), and their F_1 hybrid were used to examine fused vein and normal pollen tube growth. Eight plants of each line were raised in the UNH greenhouse during the Spring of 1992. All plants received the same cultural treatments: 12 inch pots, Promix soil-less medium, periodic feeding with soluble fertilizer, and pest control.

Pollination Treatments. Beginning 15 March and continuing for two weeks, controlled co-pollinations were made using F_1 female flowers and fused vein and normal male flowers. A day prior to anthesis, male and female flowers were identified and protected from natural pollination by tieing their corollas shut. Following anthesis, flowers were gathered, opened, and placed in beakers of water at room temperature. Female pistils were partitioned with glass cover slips (22 millimeters square), which were inserted lengthwise through the stigma and style and into the upper 5 millimeters of each ovary. For treatment pollinations, the divided stigmas were saturated with fused vein pollen on one side and with normal pollen on the other. Control pollinations consisted of both sides pollinated with fused vein or normal pollen. The female flowers were closed, retied and left for 5 or 8 hours. Stigmas, styles and ovaries were then removed intact, labeled and fixed in FAA solution (90 ml 50 % ethyl alcohol, 5 ml glacial acetic acid, 5 ml 40% formaldehyde) for storage and later examination. Eight treatment and two control pollina-

tions were made for each time period.

Preparation and Examination. The halves from each individual flower were prepared and examined concurrently. After washing thoroughly in tap water, the lower 7 millimeters of the style (style fork to ovary juncture) were removed. Pins were inserted lengthwise into the outer edge of each half to provide support. Several thin longitudinal sections were sliced from the inner surface of each half containing all of the yellowish transmitting tissue of the style. Sections were placed on a glass slide, stained with aniline blue (0.005% aniline blue in 0.15 M K_2HPO_4 at pH 8.2), and gently squashed under a second glass slide. Sections were examined microscopically for the presence of pollen tubes using both visible and fluorescent light. Based on cumulative counts in each section, each style half was rated for pollen tube number at both its stigma and ovary ends: 0 for no tubes, 1 for 1-20, 2 for 21-40, and 3 for 41-60, 4 for 61-80, and 5 for 81 or more tubes. Significance of genotype, time and style position were tested with a factorial analysis of variance.

Seed Distribution Study

Plant Material. The fused vein inbred line NH2405 (J. B. Loy, Department Plant Biology, UNH, Durham, NH) was used to examine the distribution of seed set within open pollinated fruit. On 10 June 1991, a 100 foot isolation row of 50 plants was sown at the Kingman Farm in Madbury, NH. The nearest *C. pepo* planting was over 500 yards away. The plants were permitted to grow, flower and set fruit freely.

Harvest and Seed Yield. In September, plants having 4 fruit (84%) were identified. Five of these plants were then selected at random for

harvest. The order of fruit set for each plant was determined by fruit position. The acropetal blooming pattern of *C. pepo* and the limited stem structure of NH2405 insured that those fruit closest to the plant crown were set first. The fruit were labeled, harvested and stored until deseeding in October. Each fruit was sliced longitudinally in half to expose its seed cavity. The cavity was then divided into proximal and distal sections of equal size. The seed within each section was removed, cleaned, dried, and counted.

The effects of fruit section and fruit position on seed set were examined using a two-way analysis of variance. Mean comparisons were preformed using Least Significant Difference (LSD). For base line comparison, potential seed set for proximal and distal sections was determined by dissecting five preserved female flowers and counting ovules in each half of the ovary. Flowers for dissection were obtained from NH2405 plots at the Woodman Horticultural Farm, Durham, NH and were preserved in Carnoy's solution (75% ethanol, 25% glacial acetic acid).

Competitive Pollination Study

Plant Material. The fused vein inbred line NH2405, the normal inbred line NH614 (J. B. Loy, Department Plant Biology, UNH, Durham, NH), and their F_1 hybrid were used to investigate the effects of pollen competition on seed yield and fused vein inheritance. The lines were sown adjacent to one another in P field at the Woodman Horticulture Farm, Durham, NH on 28 May 1991. The plot consisted of 100 foot rows: 4 rows for each line, 50 plants per row, 2 feet between plants, and 6 feet between rows. Following droughty weather and poor stand establishment, the plot was overplanted

on 10 June. To insure sufficient plant material, a secondary plot in E2 field was utilized for one replication of treatments. The E2 plot was planted 30 May and consisted of 60 foot rows, one for each line, at the same plant and row spacing as in P field.

Pollination Treatments. From 14 July to 7 August, hand pollinations were made between 7 and 10 AM on all three lines. Female and male flowers were identified a day prior to anthesis and protected from natural pollination by tieing their corollas shut. Following anthesis, female flowers were designated at random for treatment and male flowers were collected to provide four pollen sources: fused vein (F), normal (N), F_1 hybrid (FN), and a 50:50 mixture (M) of fused vein and normal pollens. Low, medium and saturated pollen loads were applied to each line for each source.

The pollen loads were achieved using a method adapted from Winsor *et al.* (1987). For saturated pollinations, the pollen from well dehisced anthers of 2 male flowers (+5000 grains) was rubbed directly onto the stigma of a single female flower. For low and medium pollinations, well-dehisced anthers from a minimum of 10 flowers were dissected into a paper cup and stirred with a glass rod to dislodge their pollen. A cardboard stick, Johnson and Johnson Q-tip cut in half, was used to collect and apply pollen. A thin and uniform pollen layer on the bottom and lower 2 millimeters of the cut end formed a single application. Counts of 35 applications onto glass slides yielded on average 233 $\pm$ 35 pollen grains per application. Two applications per stigma were used for the low pollen load and 4 applications were used for the medium load. The low level was selected to minimize pollen competition and maximize seed yield; the

number of pollen grains approximated the highest seed yields obtained from previous inheritance study pollinations. Medium and saturated loads were chosen to respectively double and maximize competition. The 50:50 mixture treatments were accomplished by dividing applications equally between fused vein and normal pollen.

As near a complete replication of pollinations was made each day as determined by the availability of female and male flowers. By the end of the pollination period fifteen replications for each source at each load were set and developing on each line. Twenty open pollinated fruit were permitted to mature per line; all other developing fruit were removed to limit fruit set to 2-3 fruit per plant.

Seed Yield. Fruit were harvested in mid September following a minimum of 40 days maturation. Hurricane Bob, a late infestation of squash bugs, and severe fruit rot reduced the number and quality of fruit per treatment. Only intact and slightly rotten fruit were harvested. Each fruit was deseeded individually, and its seed was cleaned, dried and counted. Seed counts from rotten fruit were included in analysis only if the total number of normally developed seeds could be determined. Analysis of variance for a completely random design with unequal subsamples was used to test for significant treatment effects. Mean comparisons for pollen source, pollen source within pollen load, and pollen load within pollen source were preformed using Least Significant Difference (LSD).

Fused Vein Recovery. Pollinations that generated F_2 and testcross populations were examined for fused vein recovery. In three cycles of growouts from October 1991 to April 1992, 30 seeds from each pollination

(19 F_1 x F_1, 13 F_1 x mixed, 7 fused vein x F_1, and 13 fused vein x mixed) were sown in 6 inch pots in the UNH greenhouse. Only seed from unrotten fruit were used in order to maximize germination. Resulting plants were grown until the tenth leaf stage and scored for leaf phenotype. Progeny were designated fused vein if they exhibited continual fused vein leaf production by the tenth leaf. All other plants were considered normal.

Chi square analyses were performed using one and two gene models for determining expected ratios. The frequencies of fused vein progeny were determined and analyzed using the Kruskal-Wallis Test and Kolmogorov-Smironov two sample comparisons. Angularly transformed frequency data was used for regression analysis. Pollen loads were assigned numeric values to represent their relative magnitudes: 1 for low, 2 for medium and 10 for saturated.

Plant Growth Study

Plant Material. The fused vein inbred line NH2405, the normal inbred line NH614 (J. B. Loy, Department Plant Biology, UNH, Durham, NH), their F_1 hybrid, F_2 generation, and fused vein backcross were used to examine the influence of the fused vein trait on plant growth. On 22 January 1992 in the UNH greenhouse, eight 12 inch pots were sown with 2 seeds each, for both the inbreds and F_1 hybrid. Twelve pots were sown for both the F_2 and backcross generations. After one week, each pot was thinned to one plant. In the third week, eight plants were selected for both the F_2 and backcross generations to achieve a balance of phenotypes (F_2: 3 fused vein and 5 normal; backcross: 4 fused vein and 4 normal). All plants received the same cultural treatments, including Promix soil-less medium, periodic

feeding with water soluble fertilizer and pest control. Emerging lateral stems were removed biweekly in order to compare absolute apical growth.

Growth Measurements. Beginning with the second week of growth, the number of expanded leaves, the total leaf area, and leaf phenotypes were determined for each plant at weekly intervals for four weeks. A leaf was considered expanded once it had unfolded and reached a width of 2.5 centimeters. Total leaf area was calculated as the sum of individual leaf area estimates. Individual estimates were determined by multiplying a blade's greatest width by its length from tip to petiole. Accumulated leaf numbers and areas for the different genotypes were compared using regression analysis.

The method for leaf area estimation was derived by sampling twenty leaves ranging in size and genotype at the conclusion of the study. They were measured, removed and photocopied. The photocopy images were cut from paper and weighed. An area by weight ratio for the paper was used to convert image weights into leaf areas. The photocopy areas were compared to width by length estimates using regression analysis. An R^2 value of .9938 indicated multiplication without a correction factor was an adequate method for leaf area determination.

Results

In situ Pollen Tube Growth Study

Pollen tube growth scores, means and standard deviations are presented in Table 5.1. The four control blossoms showed similar pollen tube growth between their halves regardless of pollen source indicating no partition effect. Only one treatment blossom showed no tube growth what so ever; it was judged aberrant and four missing data points were estimated following Steel and Torrie (1980).

Analysis of variance revealed significant genotype, time and position effects for tube growth with no interaction of factors (Table 5.2). Normal pollen tubes were more abundant then fused vein tubes in both style sections and across both time periods, particularly for the eight hour treatment (Figure 5.1). Regardless of genotype, pollen tube

Table 5.1. Pollen tube growth scores[a], means and standard deviations.

	Treatment[b]					Treatment			
Blossom	5FRS	5FRO	5NLS	5NLO	Blossom	8FRS	8FRO	8NLS	8NLO
1	4	3	5	5	9	3	2	2	3
2	1	0	1	0	10	4	3	5	5
3	3	2	3	2	11	3	1	4	4
4	3	2	4	3	12	4	3	4	3
5	3	3	4	3	13	2	1	4	3
6	4	3	3	2	14	5	4	5	5
7	2	0	2	0	15	3	2	4	3
8[c]	3.1	0.3	3.9	1.1	16	3	3	3	4
mean	2.88	1.66	3.23	2.00		3.37	2.37	3.88	3.75
std	0.92	1.27	1.19	1.57		0.86	0.99	0.93	0.83

Control	5RS	5RO	5LS	5LO	Control	8RS	8RO	8LS	8LO
Fused	3	2	3	2	Fused	3	2	3	2
Normal	4	3	4	3	Normal	4	4	5	4

[a] 0 = 0, 1 = 1-20, 2 = 21-40, 3 = 41-60, 4 = 61-80, 5 > 81 pollen tubes
[b] 5= five hours, 8 = eight hours, F = fused vein pollen, N = normal pollen, R = right half, L = left half, S = stigma end, O = ovary end.
[c] estimated missing data points.

number increased over time and diminished with distanced traveled. Normal tube densities, however, were not significantly different between the style ends for the eight hour treatment. In two different five hour pollinations, fused vein and normal pollen tubes failed to reach the base of the style; in both instances, however, normal tubes outgrew fused vein tubes (Figure 5.2).

Table 5.2. Analysis of variance for pollen tube growth by genotype, hour and section.

Source	df	SS	MS	F
Treatments	7	36.87	5.27	3.55 *** [a]
Genotype (A)	1	6.58	6.58	4.43 *
Hour (B)	1	12.78	12.78	8.61 ***
Section (C)	1	12.82	12.82	8.64 ***
AB	1	0.75	0.75	0.50 ns
AC	1	1.40	1.40	0.95 ns
BC	1	1.76	1.76	1.18 ns
ABC	1	0.78	0.78	0.53 ns
Error	52	77.18	1.48	
Total	59	114.04		

[a] *** = $P < 0.005$, ** = $P < 0.010$, * = $P < 0.05$, ns = not significant

Seed Distribution Study

Two-way analysis of variance indicated significant section and position effects on seed yield in open pollinated fused vein fruit (Table 5.3). Mean comparisons revealed a significantly higher seed yield in the proximal section for all fruit, with approximately a three fold increase over the distal section (Table 5.4). A trend of increasing yield was also observed for fruit position; fruit furthest from the crown, and therefore last to be set, had the highest average seed yields. The average yield per fruit was 170 $\pm$ 42 seeds. This was lower than expected, approximately one half the open pollinated yield obtained in the pollen competition

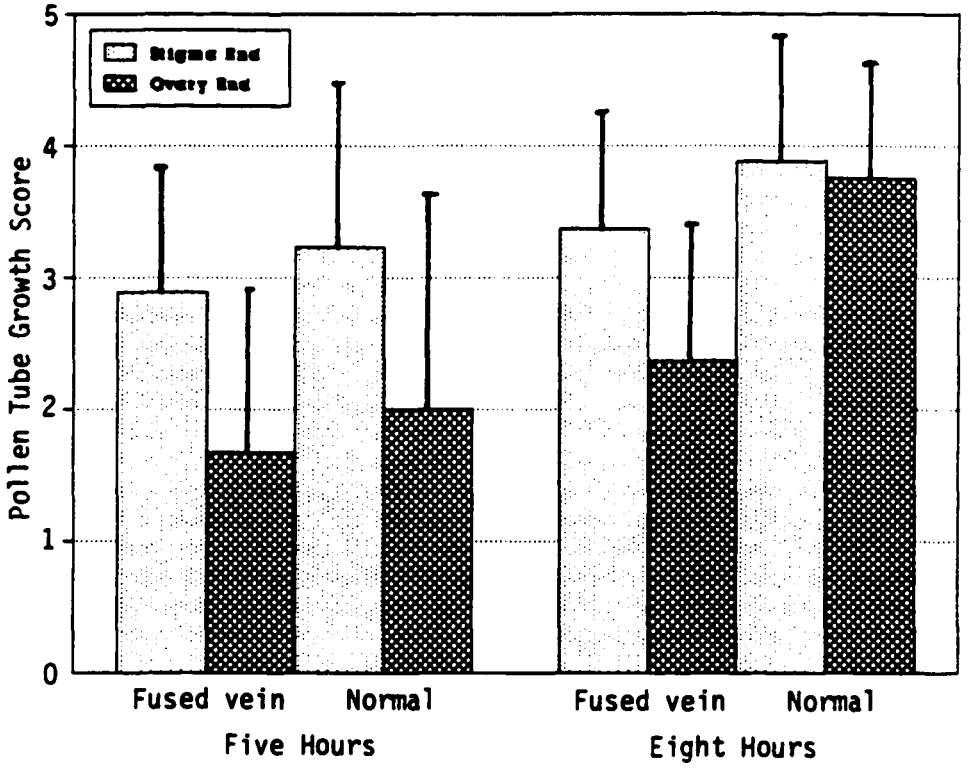


Figure 5.1. Comparison of pollen tube growth by genotype, time and style section.

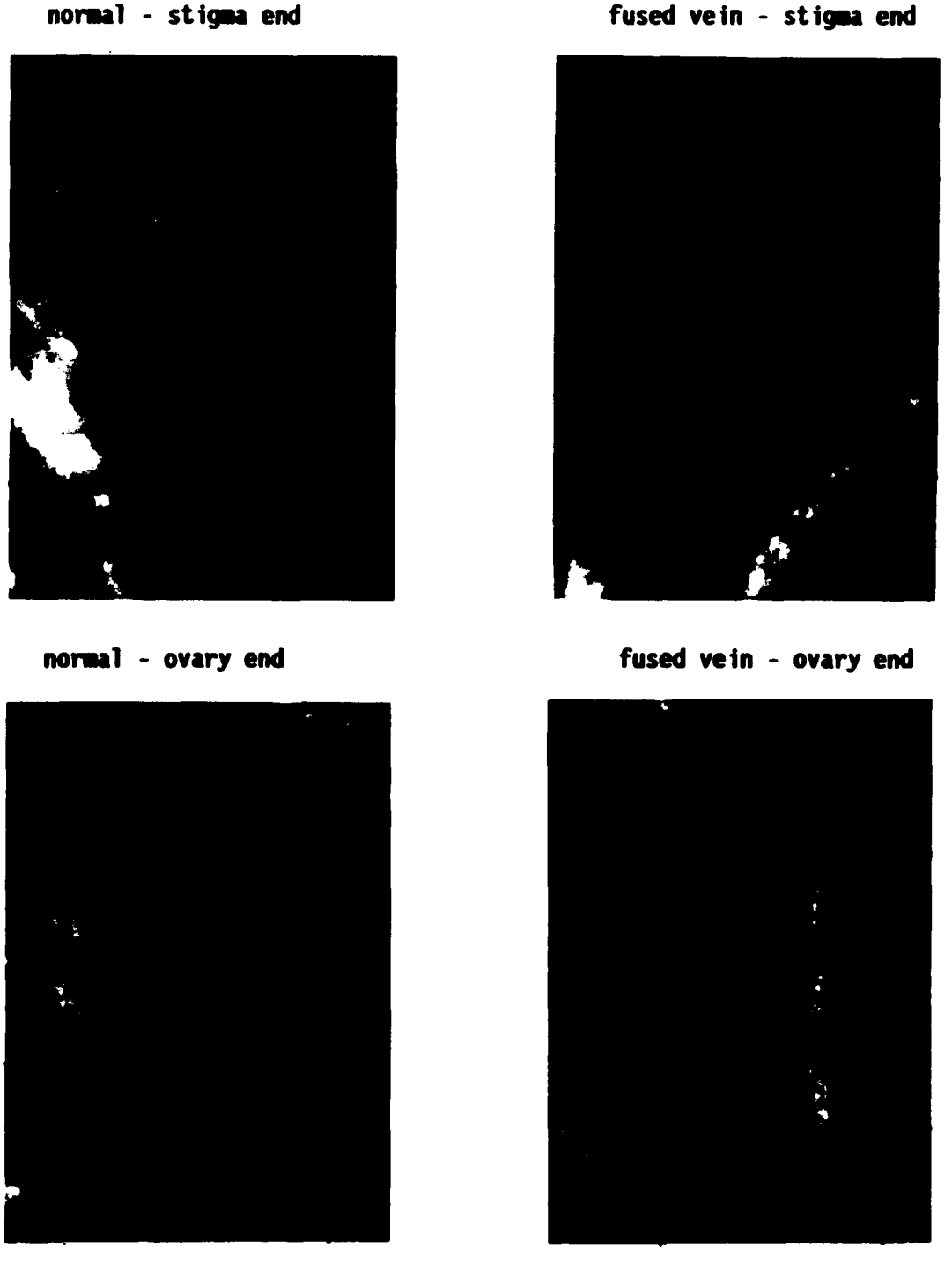


Figure 5.2. Fused vein and normal pollen tube growth in paired style halves.

Table 5.3. Analysis of variance of fused vein seed yield by fruit section and position.

Source	df	SS	MS	F
Model	7	76512.17	10930.31	9.12 ***
Section	1	65853.22	65853.22	191.16 ***
Position	3	9625.47	3208.49	9.31 *
Section/Position	3	1033.48	344.49	0.29 ns
Error	32	38334.80	1197.96	
Total	39	114846.97		

[x] *** = P < 0.005, ** = P < 0.010, * = P < 0.05, ns = not significant

Table 5.4. Mean comparisons for fused vein seed yield by fruit section and position.

Section	Fruit Position[z] 1st	2nd	3rd	4th	Combined
Blossom	30.6 a[y]	39.6 a	42.2 a	65.2 ab	44.4 g
Stem	98.4 b	117.4 bc	138.4 b	148.2 c	125.6 h
Combined	129.0 q	157.0 q	180.8 qr	213.4 r	

[z] Position refers to order of fruit set based on proximity to plant crown.
[y] Different letters denote significant differences between means. Pairwise LSD tests (P < 0.05) were used to compare individual fruit sections, the combined fruits, and the combined sections.

study. The ovule counts estimated an average potential yield of 553 ± 63 seed per fruit with an even distribution of seed between proximal and distal sections, 282 ± 36 and 271 ± 27 respectively.

Competitive Pollination Study

Seed Yields. Pairwise LSD comparisons of open pollinated yields revealed significant differences (P =.05) among the three female genotypes (Table 5.5). The fused vein line yielded, on average, fewer seed per fruit then the normal line while the F_1 exhibited heterosis, out-yielding either parent. These inherent yield differences and unequal treatment replications precipitated separate analyses for each female line and each cross.

Table 5.5. Mean seed yield for open pollinated fruit.

Fruit type	Sample Number	Mean	Range	Standard Deviation
Fused Vein	18	330 a[z]	121-432	87.7
Normal	17	449 b	278-638	85.5
F_1 Hybrid	15	535 c	264-691	128.2

[z] Different letters denote significant differences between means. Pairwise LSD tests ($P < 0.05$) were used because of unequal sample numbers.

Random linear models were constructed to test the effects of pollen source and pollen load on seed yield (Table 5.6). Pollen source had a significant ($P = .05$) influence on yield for both the normal and F_1 lines and was marginally significant ($P = .10$) for the fused vein line. In contrast, pollen load did not significantly alter yield when individual crosses were combined. Pollen load was, however, highly significant ($P = .01$) for two individual crosses (F x F and N x M) and marginally significant ($P = .10$) for three other crosses (N x F_1, F_1 x F_1, and F_1 x M).

Individual effects of pollen source on seed yield were uncovered by pairwise LSD comparisons (Table 5.7). Fused vein pollen produced significantly ($P = .05$) lower average seed yields for six of the nine sets of source by load comparisons. In general, normal, F_1 and mixed pollen were not significantly different from each other. Normal pollen, however, did produce the highest average yields, particularly at the low pollen load. Combining loads within source, as suggested by the analysis of variance, corroborated the opposite effects of fused vein and normal pollen on seed yield. The combined load means also revealed, albeit not significantly, that F_1 pollen produced higher average yields then mixed pollen. Figure 5.3 illustrates the pollen source effects for the combined load means and includes open pollinated means for comparison.

Table 5.6. Analyses of variance for pollen source and load effects on fused vein, normal, and F_1 seed yield.

	Fused Vein Fruit				Normal Fruit				F_1 Fruit			
Source	df	SS	MS	F	df	SS	MS	F	df	SS	MS	F
Combined Pollen Sources												
Model	11	163553.8	14868.5	2.68 **[d]	11	274101.6	24918.3	3.74 ***	11	503427.1	45766.1	4.93 ***
Source	3	76581.9	25527.3	2.24[a] *	3	170761.7	56920.6	4.29[b] **	3	364856.4	121618.8	5.14[c] ***
Load/Source	8	86971.9	10871.5	1.96 ns	8	103339.9	12917.5	1.94 ns	8	138570.7	17321.3	1.86 ns
Error	43	238729.8	5551.9		43	286439.5	6661.4		78	724495.8	9288.4	
Total	54	402283.6			54	834642.8			89	1227922.9		
Individual Pollen Sources												
Fused Vein Pollen												
load	2	37281.2	18640.6	11.85 ***	2	6242.0	3121.0	2.73 ns	2	14194.2	7097.1	1.52 ns
error	9	14155.5	1572.8		7	7996.0	1142.3		19	88601.7	4663.3	
total	11	51436.7			9	14238.0			21	102795.9		
Normal Pollen												
load	2	11400.7	5700.4	0.58 ns	2	31455.6	15727.8	1.16 ns	2	12403.0	6201.5	0.39 ns
error	15	146339.7	9756.0		14	190557.4	13611.2		16	254877.1	15929.8	
total	17	157740.4			16	222013.0			18	267280.1		
F_1 Pollen												
load	2	22254.1	11127.1	3.98 ns	2	34938.0	17469.0	3.23 *	2	62396.9	31198.5	3.24 *
error	4	11180.8	2795.2		15	81090.0	5406.0		32	308106.7	9628.3	
total	6	33434.9			17	116028.0			34	370503.6		
Mixed Pollen												
load	2	16036.0	8018.0	1.79 ns	2	30704.3	15362.2	15.81 ***	2	49576.6	24788.3	3.74 *
error	15	67053.8	4470.3		7	6796.1	970.9		11	72910.3	6628.2	
total	17	83089.8			9	37500.4			13	122486.9		

[a] Adjusted error term for unequal sample size: 23669.73, effective df: 40.
[b] Adjusted error term for unequal sample size: 11380.43, effective df: 23.
[c] Adjusted error term for unequal sample size: 13254.14, effective df: 37.
[d] ns = not significant, * = $P < 0.10$, ** = $P < 0.05$, *** = $P < 0.01$.

Table 5.7. Mean seed yields for fused vein, normal and F_1 fruit by pollen source.

Female and Pollen Source	Pollen Load			All Loads Combined
	low	medium	saturated	
Fused Vein Female				
Fused Vein	105 a[z]	160 a	241 a	169 a
Normal	298 b	281 b	237 a	271 b
F_1 Hybrid	159 ab	286 b	216 a	240 b
50:50 Mix	213 b	262 b	196 a	233 b
Open Pollinated				330 c[y]
Normal Female				
Fused Vein	131 a	186 a	137 a	155 a
Normal	258 c	283 a	356 c	312 c
F_1 Hybrid	207 b	273 a	316 bc	271 bc
50:50 Mix	175 b	178 a	289 b	221 b
Open Pollinated				449 d
F_1 Hybrid Female				
Fused Vein	276 a	219 a	286 a	273 a
Normal	378 a	446 b	410 b	416 bc
F_1 Hybrid	316 a	389 b	452 b	428 c
50:50 Mix	291 a	361 b	453 b	356 b
Open Pollinated				535 d

[z] Different letters in the same column within female denote significant differences between means. Pairwise LSD tests ($P < 0.05$) were used due to the unequal number of samples for each treatment.
[y] Significant at $P < 0.10$

Table 5.8. Mean seed yield for fused vein, normal and F_1 fruit by pollen load.

Female and Pollen Load	Pollen Source			
	Fused Vein	Normal	F1 Hybrid	50:50 Mix
Fused Vein Female				
low	105 a[z]	298 a	159 a	213 a
medium	160 a	281 a	286 b	262 a
saturated	241 b	237 a	216 ab	196 a
Normal Female				
low	131 a	258 a	207 a	175 a
medium	186 b	283 a	273 a	178 a
saturated	137 ab	356 a	316 b	289 b
F_1 Hybrid Female				
low	276 a	378 a	316 a	291 a
medium	219 a	446 a	389 ab	361 ab
saturated	286 a	410 a	452 b	453 b

[z] Different letters in the same column within female denote significant differences between means. Pairwise LSD tests ($P < 0.05$) were used due to the unequal number of samples for each treatment.

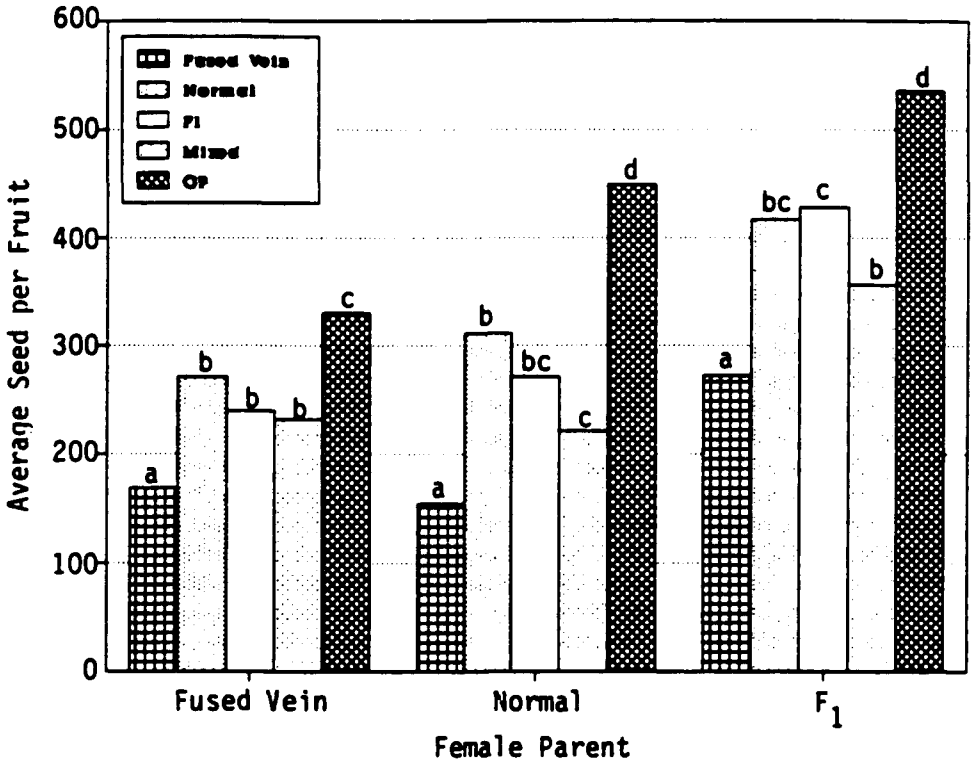


Figure 5.3. Effect of pollen source on average seed yield for fused vein, normal and F_1 fruit.

Although the analyses of variance indicated no significant pollen load effect, LSD comparisons showed a decrease in yield at the low pollen load for a majority of crosses involving fused vein, F_1 and mixed pollen (Table 5.8). In contrast, crosses with normal pollen showed no significant decreases, suggesting a weakness of the fused vein gametophyte. Windsor and colleagues (1987), using a low seed yielding zucchini, 'Black Beauty', obtained ten and six fold reductions in seed yield using pollen loads half of and equal to this investigation's low load. Together, the results suggest that gametophytic genes determine, in part, the pollen load threshold for maximum seed.

Fused Vein Recovery. The frequencies of fused vein progeny obtained in F_2 and testcross populations varied considerably (Table 5.9). As in previous inheritance studies (Chapter VI), chi square analysis showed fit to both single and double recessive gene models for both F_2 (Table 5.10) and testcross populations (Table 5.11). However, fit was also associated with pollen load. All but one single gene fit occurred at the low or medium pollen loads. Single gene fit predominated at the low load for backcross populations and at the medium load for F_2 populations. Failure to recover fused vein individuals occurred only at the saturated load for F_2 populations generated with F_1 pollen.

The skewed distribution of F_2 fused vein frequencies obtained with saturated F_1 pollen precipitated nonparametric evaluation of the data. Kruskal-Wallis analysis revealed a significant pollen load effect but no significant difference between pollen sources for both the F_2 and testcross populations (Table 5.12). Kolmogorov-Smirnov two sample comparisons, with

Table 5.9. Means, ranges, and standard deviations for fused vein frequency in F_2 and testcross populations by pollen load.

Pollination, Pollen load	Sample*	Mean	Range	Standard Deviation
F_1 x F_1				
low	3	.118	.067-.182	.055
medium	6	.127	.021-.239	.088
saturated	10	.038	.000-.152	.052
F_1 x Mixed				
low	5	.132	.057-.244	.067
medium	5	.178	.087-.227	.061
saturated	3	.063	.045-.073	.012
Fused Vein x F_1				
low	2	.576	.483-.667	.134
medium	4	.415	.346-.467	.054
saturated	1	.200		
Fused Vein x Mixed				
low	3	.456	.300-.552	.136
medium	8	.349	.214-.552	.107
saturated	3	.248	.200-.267	.044

* Number of hand pollinated fruit from which 30 seeds were grown.

separate or combined pollen sources for both population groups, showed significantly lower fused vein frequencies for the saturated pollen load when compared to the low and medium loads (Table 5.13). The low and medium loads were only significantly different from each other in the testcross populations.

The absence of a pollen source effect is consistent with the proposed single recessive gene model for fused vein inheritance as discussed in Chapter IV. This model allows the presumption that F_1 and mixed pollen treatments in the competition study were genetically equivalent, 50% recessive (f) and 50% dominant gametes (F), resulting in similar patterns of inheritance and no source effect. With respect to the double recessive model, interpretation of the absent source effect is confounded and inconclusive. The occurrence of single gene ratios among both F_2 and testcross

Table 5.10. Fused vein segregation for F_2 populations derived from (NH2405 x NH614) and F_1 and mixed pollen sources.

Cross	Pollen[a] Load	Phenotype[b] N	F	Freq. Fused	X^2[c] 3:1	P	X^2[c] 15:1	P
(NH2405 x NH614) x F_1 Pollen								
91-1	low	36	8	0.18	1.09	.50-.25	10.69	<.005
91-2	low	42	5	0.11	5.17	.02-.01	1.54	.25-.10
91-3	low	42	3	0.07	8.07	<.005	0.01	.95-.90
91-4	med	35	11	0.24	0.03	.90-.75	24.49	<.005
91-5	med	33	8	0.20	0.66	.50-.25	12.31	<.005
91-6	med	34	7	0.17	1.37	.25-.10	8.20	<.005
91-7	med	31	3	0.09	4.75	.05-.02	0.38	.75-.50
91-8	med	42	2	0.05	9.82	<.005	0.22	.75-.50
91-9	med	47	1	0.02	13.44	<.005	1.42	.25-.10
91-10	sat	39	7	0.15	2.35	.25-.10	6.31	.02-.01
91-11	sat	44	4	0.08	7.11	.01-.005	0.36	.75-.50
91-12	sat	44	4	0.08	7.11	.01-.005	0.36	.75-.50
91-13	sat	46	2	0.04	11.11	<.005	0.35	.75-.50
91-14	sat	47	1	0.02	13.44	<.005	1.42	.25-.10
91-15	sat	48	0	0.00	16.00	<.005	3.20	.10-.05
91-16	sat	48	0	0.00	16.00	<.005	3.20	.10-.05
91-17	sat	28	0	0.00	9.33	<.005	1.87	.25-.10
91-18	sat	47	0	0.00	15.67	<.005	3.13	.10-.05
91-19	sat	45	0	0.00	15.00	<.005	3.00	.10-.05
(NH2405 x NH614) x Mixed Pollen								
91-1	low	34	11	0.24	0.01	.95-.90	25.42	<.005
91-2	low	35	5	0.13	3.33	.10-.05	2.67	.25-.10
91-3	low	42	6	0.13	4.00	.05-.02	3.20	.10-.05
91-4	low	43	5	0.10	5.44	.02-.01	1.42	.25-.10
91-5	low	33	2	0.06	6.94	.01-.005	0.02	.90-.75
91-6	med	34	10	0.23	0.12	.75-.50	20.39	<.005
91-7	med	35	10	0.22	0.19	.75-.50	19.59	<.005
91-8	med	30	8	0.21	0.32	.75-.50	14.21	<.005
91-9	med	36	6	0.14	2.57	.25-.10	4.63	.05-.02
91-10	med	42	4	0.09	6.52	.02-.01	0.47	.50-.25
91-11	sat	40	3	0.07	7.45	.01-.005	0.04	.90-.75
91-12	sat	38	3	0.07	6.84	.01-.005	0.08	.90-.75
91-13	sat	42	2	0.05	9.82	<.005	0.22	.75-.50

[a] Approximate Pollen Loads: low, 460 pollen grains; med, 920 pollen grains; sat, +5000 pollen grains.
[b] Phenotypes: N normal, F fused vein.
[c] X^2 df = 1.

Table 5.11. Fused vein segregation for testcross populations derived from NH2405 and F_1 and mixed pollen sources.

Cross	Pollen[a] Load	Phenotype[b] N	F	Freq. Fused	X^{2c} 1:1	P	X^{2c} 3:1	P
NH2405 x F_1 Pollen								
91-1	low	9	18	0.67	3.00	.50-.25	25.00	<.005
91-2	low	15	14	0.48	0.03	.02-.01	8.38	.25-.10
91-3	med	16	14	0.47	0.13	<.005	7.51	.95-.90
91-4	med	16	13	0.45	0.31	.90-.75	6.08	<.005
91-5	med	18	12	0.40	1.20	.50-.25	3.60	<.005
91-6	med	17	9	0.35	2.46	.25-.10	1.28	<.005
91-7	sat	24	6	0.20	10.80	.05-.02	0.40	.75-.50
NH2405 x Mixed Pollen								
91-1	low	13	16	0.55	0.31	.75-.50	14.08	<.005
91-2	low	14	15	0.52	0.03	.90-.75	11.05	<.005
91-3	low	21	9	0.30	4.80	.05-.02	0.40	.75-.50
91-4	med	13	16	0.55	0.31	.75-.50	14.08	<.005
91-5	med	16	13	0.45	0.31	.75-.50	6.08	.02-.01
91-6	med	18	10	0.36	2.29	.25-.10	1.71	.25-.10
91-7	med	18	9	0.33	3.00	.10-.05	1.00	.50-.25
91-8	med	20	9	0.31	4.17	.05-.02	0.56	.50-.25
91-9	med	20	9	0.31	4.17	.05-.02	0.56	.50-.25
91-10	med	22	8	0.27	6.53	.02-.01	0.04	.90-.75
91-11	med	22	6	0.21	9.14	<.005	0.19	.75-.50
91-12	sat	21	8	0.28	5.83	.02-.01	0.10	.90-.75
91-13	sat	22	8	0.27	6.53	.02-.01	0.04	.90-.75
91-14	sat	24	6	0.20	10.80	<.005	0.40	.75-.50

[a] Approximate Pollen Loads: low, 460 pollen grains; med, 920 pollen grains; sat, +5000 pollen grains.
[b] Phenotypes: N normal, F fused vein.
[c] X^2 df = 1.

populations indicates that the F_1 employed in the competition study had either a 33.3% chance of being all heterozygote single recessives (Ffvv) or a 66.6% of being an even mixture of heterozygote single recessives (Ffvv) and double heterozygotes (FfVv). In the former case, F_1 and mixed pollen would have been equivalent resulting in no source effect. In the latter case, with random selection of male flowers, the F_1 pollen treatments would have had on average 37.5% double recessive gametes (fv) as compared to 50% for the mixed pollen treatments. Consequently, a source

Table 5.12. Kruskal-Wallis analyses of fused vein recovery.[a]

Pollen Source	Pollen Load	Rank Sum	n	H	D	Adjusted H	df	P
F_2 Populations								
Subgroups								
F_1	low	41.0	3	14.54	0.9956	14.61	5	.025 - .010
F_1	med	81.0	6					
F_1	sat	245.5	10					
mixed	low	60.5	5					
mixed	med	38.0	5					
mixed	sat	62.5	3					
Pollen Sources - Loads Combined								
F_1	both	367.5	19	3.29	0.9956	4.31	1	.10 - .05
mixed	both	160.5	13					
Pollen Loads - Sources Combined								
both	low	81.0	8	7.87	0.9956	7.91	2	.025 - .010
both	med	119.0	11					
both	sat	308.0	13					
Testcross Populations								
Subgroups								
F_1	low	6.0	2	11.53	0.9968	11.57	5	.05 - .025
F_1	med	33.5	4					
F_1	sat	20.5	1					
mixed	low	21.5	3					
mixed	med	95.5	8					
mixed	sat	54.0	3					
Pollen Source - Loads Combined								
F_1	both	60.0	7	1.61	0.9968	1.61	1	.25 - .10
mixed	both	171.0	14					
Pollen Loads - Sources Combined								
both	low	27.5	5	9.99	0.9968	10.02	2	.01 - .005
both	med	129.0	12					
both	sat	74.5	4					

[a] Adjusted H value greater then $X^2_{[.05]}$ rejects the null hypothesis that the samples tested come from statistically equivalent populations.

Table 5.13. Mean fused vein frequencies for F_2 and backcross populations by pollen load and pollen source.

Pollen Load	F_2s			Backcrosses		
	F_1	Mixed	Combined	F_1	Mixed	Combined
low	.118 a[z]	.132 ab	.128 a	.576 a	.456 a	.503 a
medium	.127 a	.178 a	.151 a	.415 b	.349 ab	.372 b
saturated	.038 b	.063 b	.043 b	.200 c	.248 b	.235 c

[z] Different letters in the same column denote significantly different means, Kolmogorov-Smirnov Ranked Two Sample Tests ($P < 0.05$).

effect should have been observed among the testcross and perhaps the F_2 populations. The mixture of F_1 genotypes obscures the F_2 expectation because it also affects female gamete production.

The variability of fused vein recovery, few replications among treatments, and the absence of a pollen source effect prompted the combination of F_1 and mixed pollen source data for regression analysis. Multiple regressions were performed, using angular transformation, to relate fused vein frequency (FF) to pollen load (PL). The best fit models for the F_2 and backcross data were: $\arcsin (FF)^{0.5} = 24.18 - 1.50(PL)$ and $\arcsin (FF)^{0.5} = 42.22 - 1.39(PL)$, respectively. Fused vein frequency exhibited a negative linear relationship to pollen load for both population groups (Figure 5.4). Their slopes were not significantly different ($t = .052$, $df = 49$), indicating differential male gametophyte performance was the same for both population groups. The models, however, accounted for only a small portion of the variation in fused vein frequency (F_2: $R^2 = 0.36$ and BC: $R^2 = 0.45$).

Plant Growth Study

Both leaf number (LN) and total leaf area (LA) increased logarithmically with time for each genotype. Following logarithmic transformation, simple linear regression models fit both growth measurements with high correlation for all genotypes (Tables 5.14, 5.15 and 5.16). The slopes did not differ significantly across genotypes for both leaf number and area indicating a common growth rate for all genotypes.

Comparison of the fused vein, normal, and F_1 regression lines is displayed in Figure 5.5. Although the fused vein inbred produced fewer

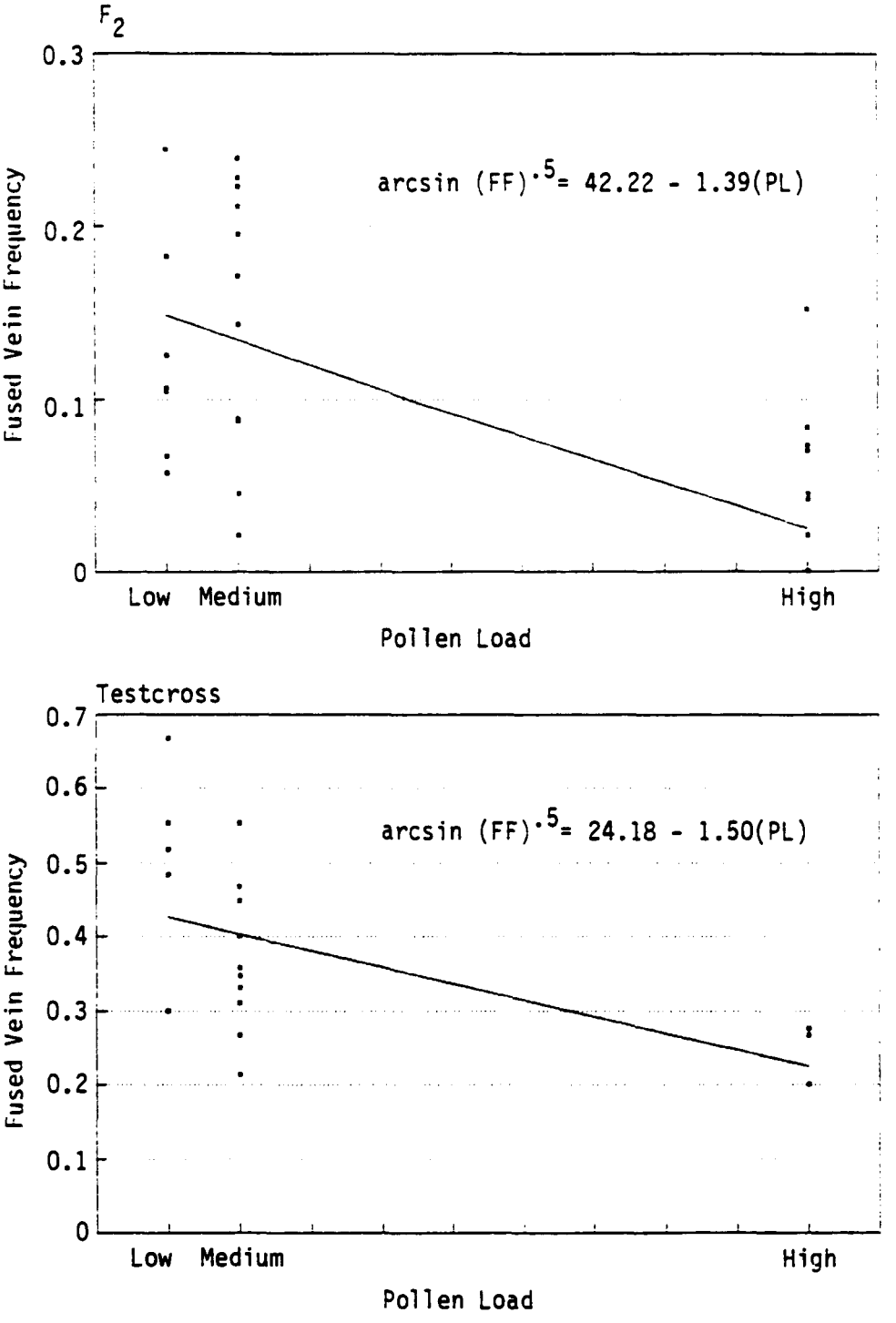


Figure 5.4. Fused vein recovery in F_2 and testcross generations by pollen load.

leaves then the normal inbred, the leaves were larger and resulted in the same total leaf area. The F_1 displayed heterosis; it produced the same number of leaves as the normal line, but with a larger size, yielding a higher total leaf area. Comparison of the fused vein and normal phenotypes in the F_2 (Figure 5.6) and backcross generations (Figure 5.7) revealed no significant differences in leaf number or total leaf area between the phenotypes. A summary of means, ranges and standard deviations for leaf number and leaf area are provided in Tables 5.17 and 5.18.

Table 5.14. Regression models for leaf number and leaf area.

Genotype (Phenotype)	Model[a]	Error Slope	Error Intercept	R^2
Leaf Number				
Fused Vein	log(LN) = 0.242(WG) - 0.123	0.008	0.053	0.965
Normal	log(LN) = 0.243(WG) + 0.030	0.007	0.045	0.975
F_1	log(LN) = 0.235(WG) + 0.018	0.005	0.032	0.986
F_2 (all)	log(LN) = 0.238(WG) - 0.010	0.006	0.041	0.978
F_2 (fused)	log(LN) = 0.246(WG) - 0.043	0.016	0.061	0.960
F_2 (normal)	log(LN) = 0.233(WG) + 0.009	0.005	0.024	0.992
F(FN) (all)	log(LN) = 0.229(WG) - 0.021	0.010	0.061	0.950
F(FN) (fused)	log(LN) = 0.225(WG) - 0.014	0.014	0.061	0.952
F(FN) (normal)	log(LN) = 0.233(WG) - 0.027	0.014	0.063	0.952
Leaf Area				
Fused Vein	log(LA) = 0.441(WG) + 1.363	0.014	0.090	0.969
Normal	log(LA) = 0.437(WG) + 1.396	0.014	0.088	0.971
F_1	log(LA) = 0.475(WG) + 1.334	0.014	0.089	0.974
F_2 (all)	log(LA) = 0.493(WG) + 1.133	0.015	0.092	0.975
F_2 (fused)	log(LA) = 0.499(WG) + 1.126	0.026	0.102	0.973
F_2 (normal)	log(LA) = 0.490(WG) + 1.137	0.018	0.090	0.976
F(FN) (all)	log(LA) = 0.463(WG) + 1.367	0.014	0.090	0.973
F(FN) (fused)	log(LA) = 0.470(WG) + 1.309	0.017	0.077	0.981
F(FN) (normal)	log(LA) = 0.456(WG) + 1.424	0.021	0.093	0.972

Comparison of Regression Slopes

	Leaf Number				Leaf Area			
Source	df	SS	MS	F	df	SS	MS	F
Slopes	8	0.123	0.015	0.28 ns[b]	8	0.009	0.0011	0.0013 ns
Error	18	0.973	0.054		18	15.648	0.8693	

[a] LN = leaf number, LA = leaf area, WG = weeks of growth
[b] ns = not significant

Table 5.13. Analyses of variance for leaf number by genotype.

Source	Fused Vein df	SS	MS	F	Normal df	SS	MS	F	F_1 df	SS	MS	F
Weeks	3	2.360	0.787	360.36*[a]	3	2.413	0.804	1500.71*	3	2.229	0.743	1080.35*
Regression	1	2.334	2.334	1128.75*	1	2.367	2.367	4415.64*	1	2.218	2.218	3224.99*
Deviations	2	0.026	0.013	6.17*	2	0.046	0.023	43.25*	2	0.011	0.006	8.02*
Error	29	0.060	0.002		29	0.016	0.001		29	0.020	0.001	
Total	32	2.419			32	2.429			32	2.249		

Source	F_2 (all) df	SS	MS	F	F_2 (fused vein) df	SS	MS	F	F_2 (normal) df	SS	MS	F
Weeks	3	2.268	0.756	491.96*	3	0.917	0.306	102.42*	3	1.359	0.453	844.59*
Regression	1	2.262	2.262	829.82*	1	0.906	0.906	166.77*	1	1.358	1.358	2119.33*
Deviations	2	0.005	0.003	1.77ns	2	0.011	0.005	1.82ns	2	0.001	0.001	1.19ns
Error	29	0.045	0.002		9	0.027	0.003		17	0.009	0.001	
Total	32	2.312			12	0.943			20	1.368		

Source	F(FN) (all) df	SS	MS	F	F(FN) (fused vein) df	SS	MS	F	F(FN) (normal) df	SS	MS	F
Weeks	3	2.108	0.703	197.02*	3	1.017	0.339	94.07*	3	1.092	0.364	89.24*
Regression	1	2.102	2.102	658.17*	1	1.012	1.012	435.47*	1	1.090	1.090	971.65*
Deviations	2	0.006	0.003	0.89ns	2	0.005	0.002	0.65ns	2	0.002	0.001	0.28ns
Error	29	0.103	0.004		13	0.047	0.004		13	0.053	0.004	
Total	32	2.211			16	1.064			16	1.145		

[a] * = significant at P of .05, ns = not significant

Table 5.14. Analyses of variance for leaf area by genotype.

Source	Fused Vein df	SS	MS	F	Normal df	SS	MS	F	F_1 df	SS	MS	F
Weeks	3	7.876	2.625	526.47*[a]	3	7.849	2.616	2332.72*	3	9.171	3.057	1130.09*
Regression	1	7.775	7.775	1559.18*	1	7.649	7.649	6820.13*	1	9.013	9.013	3331.70*
Deviations	2	0.101	0.050	10.12*	2	0.200	0.100	89.02*	2	0.158	0.079	29.28*
Error	29	0.145	0.005		29	0.033	0.001		29	0.078	0.003	
Total	32	8.021			32	7.881			32	9.250		

Source	F_2 (all) df	SS	MS	F	F_2 (fused vein) df	SS	MS	F	F_2 (normal) df	SS	MS	F
Weeks	3	9.858	3.286	778.82*	3	3.795	1.265	283.92*	3	6.066	2.022	447.77*
Regression	1	9.727	9.727	2305.26*	1	3.731	3.731	837.53*	1	5.996	5.996	1327.83*
Deviations	2	0.132	0.066	15.61*	2	0.063	0.032	7.11*	2	0.070	0.035	7.74*
Error	29	0.122	0.004		9	0.040	0.004		17	0.077	0.005	
Total	32	9.981			12	3.835			20	6.143		

Source	F(FN) (all) df	SS	MS	F	F(FN) (fused vein) df	SS	MS	F	F(FN) (normal) df	SS	MS	F
Weeks	3	8.690	2.897	719.86*	3	4.469	1.490	765.33*	3	4.223	1.408	338.48*
Regression	1	8.565	8.565	2128.64*	1	4.411	4.411	2266.24*	1	4.156	4.156	999.39*
Deviations	2	0.124	0.062	15.47*	2	0.058	0.029	14.88*	2	0.067	0.033	8.02*
Error	29	0.117	0.004		13	0.025	0.002		13	0.054	0.004	
Total	32	8.806			16	4.494			16	4.277		

a * = significant at P of .05, ns = not significant

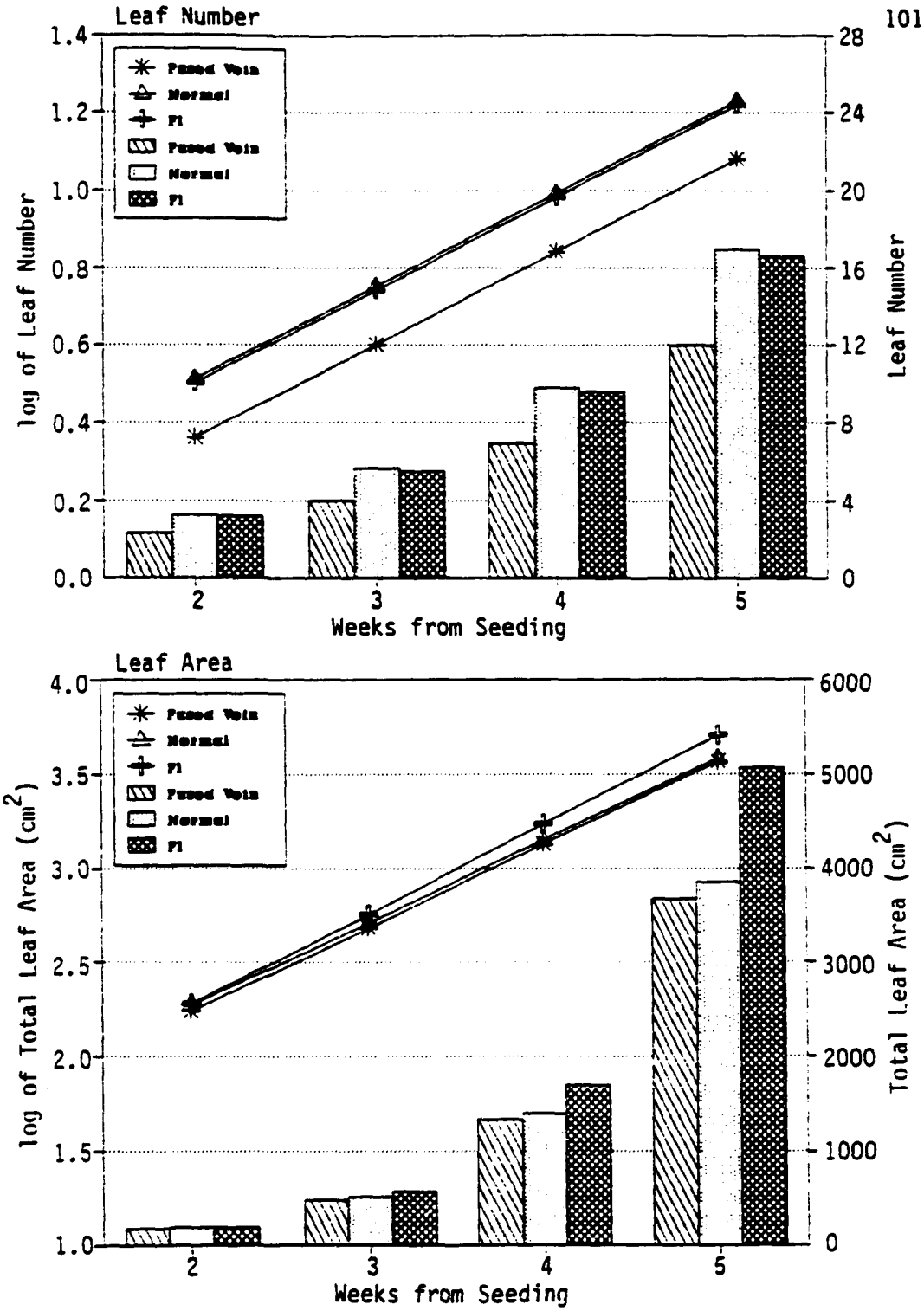


Figure 5.5. Comparison of leaf number and leaf area for fused vein, normal and F_1 lines.

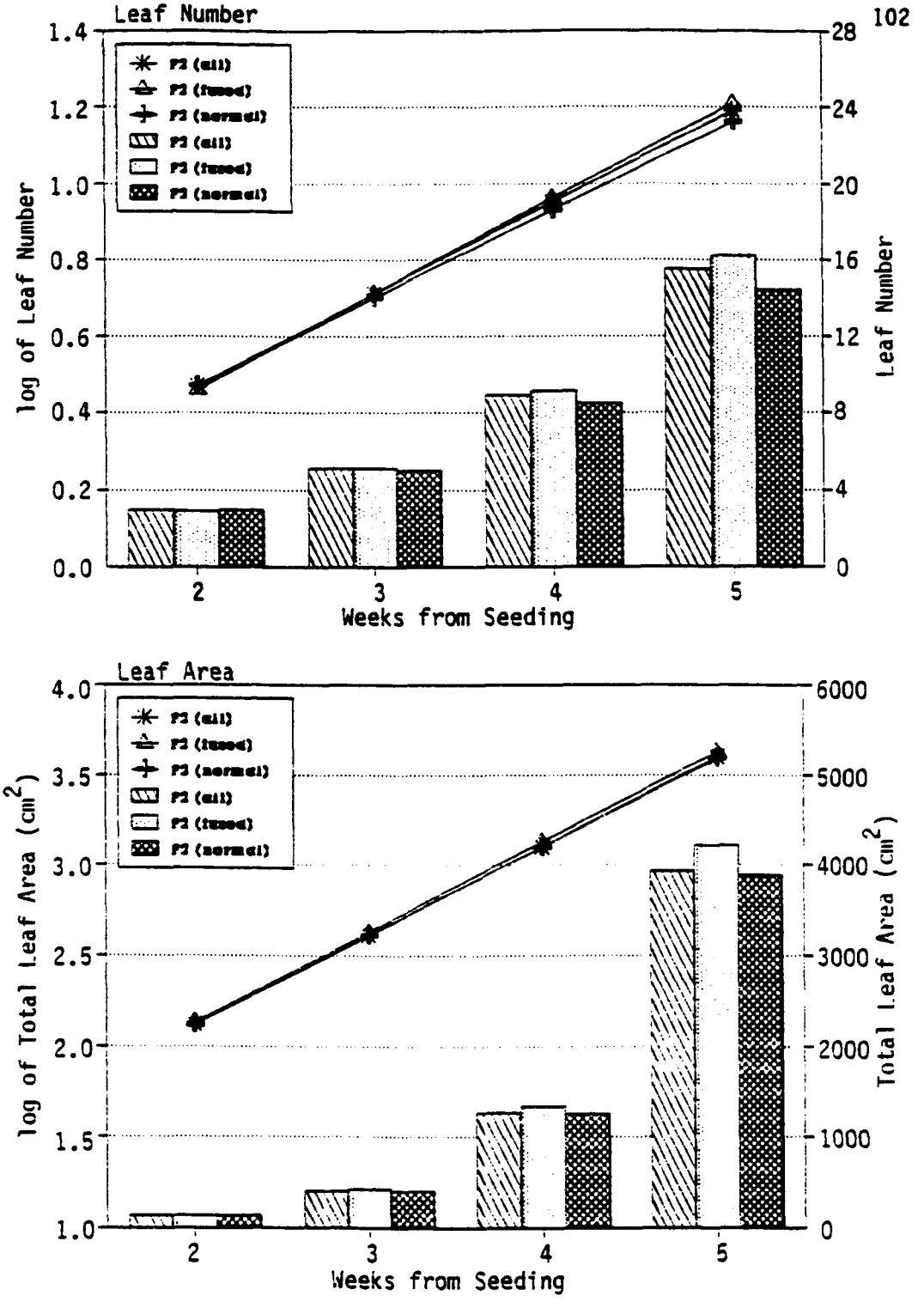


Figure 5.6. Comparison of leaf number and leaf area for fused vein and normal phenotypes in the F_2 generation.

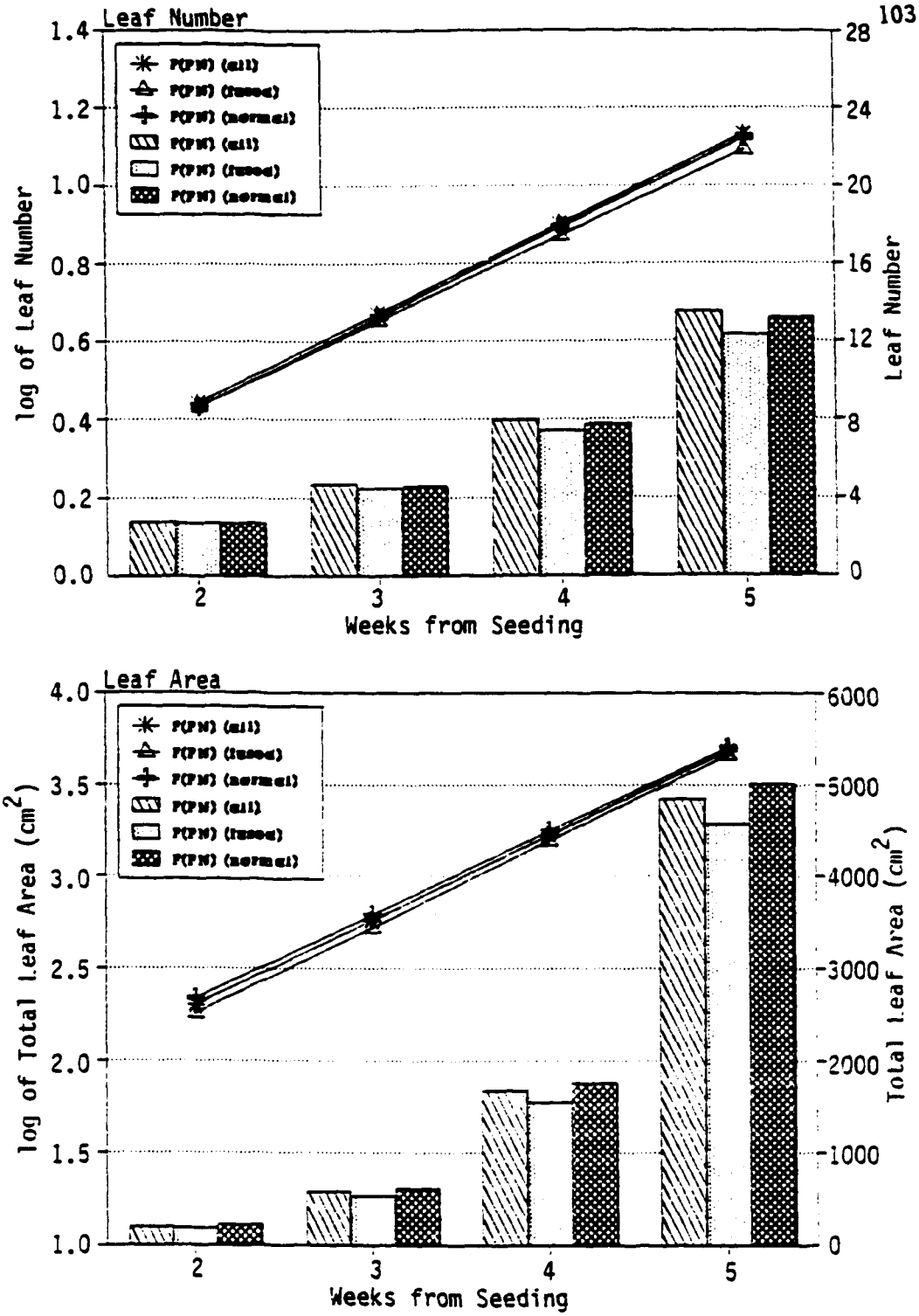


Figure 5.7. Comparison of leaf number and leaf area for fused vein and normal phenotypes in the backcross generation.

Table 5.17. Means, ranges, and standard deviations for number of leaves by week of growth.

Week	Fused Vein Mean	Range	STD	Normal Mean	Range	STD	F_1 Mean	Range	STD
2	2.3	2-3	0.43	3.0	3	0.00	3.0	3	0.00
3	4.0	4	0.00	6.4	6-7	0.48	5.4	5-6	0.48
4	7.8	7-8	0.43	10.9	10-12	0.60	9.8	9-10	0.43
5	11.5	10-13	0.86	16.3	15-17	0.66	15.0	14-16	0.87
Week	F_2 (all) Mean	Range	STD	F_2 (fused vein) Mean	Range	STD	F_2 (normal) Mean	Range	STD
2	2.9	2-3	0.33	2.7	2-3	0.47	3.0	3	0.00
3	5.1	5-6	0.33	5.3	5-6	0.47	5.0	5	0.00
4	9.1	8-10	0.60	9.3	9-10	0.47	9.0	8-10	0.63
5	14.6	13-16	0.86	14.3	4-15	0.47	14.8	13-16	0.98
Week	F(FN) (all) Mean	Range	STD	F(FN) (fused vein) Mean	Range	STD	F(FN) (normal) Mean	Range	STD
2	2.8	2-3	0.43	2.8	2-3	0.43	2.8	2-3	0.43
3	4.6	4-5	0.48	4.5	4-5	0.50	4.8	4-5	0.43
4	8.4	7-10	0.99	8.5	7-9	1.19	8.3	7-10	0.83
5	13.0	11-15	1.41	12.5	11-14	1.12	13.5	12-15	1.50

Table 5.18. Means, ranges and standard deviations for total leaf area (cm^2) by week of growth.

Week	Fused Vein Mean	Range	STD	Normal Mean	Range	STD	F_1 Mean	Range	STD
2	157	121-190	24.3	156	138-175	12.8	16	137-197	18.7
3	555	415-632	78.4	621	562-657	30.3	678	559-805	87.1
4	1551	1116-1795	230.6	1660	1414-1812	115.7	2019	1701-2418	220.0
5	3276	2638-3975	472.0	3217	2757-3554	266.7	4354	3415-4909	446.2
Week	F_2 (all) Mean	Range	STD	F_2 (fused vein) Mean	Range	STD	F_2 (normal) Mean	Range	STD
2	115	96-165	21.0	112	96-124	11.9	116	98-165	24.9
3	490	420-626	76.1	515	467-597	58.3	474	420-626	76.8
4	1458	1229-1845	197.9	1524	1350-1845	227.8	1419	1229-1698	165.5
5	3481	3039-4492	482.5	3620	3179-4492	616.6	3397	3039-3970	354.5
Week	F(FN) (all) Mean	Range	STD	F(FN) (fused vein) Mean	Range	STD	F(FN) (normal) Mean	Range	STD
2	171	137-202	21.3	154	137-172	14.2	187	166-202	13.7
3	670	560-826	101.8	608	567-686	46.2	731	560-826	105.1
4	1907	1562-2256	286.8	2059	1628-191	287.6	1755	1562-2256	125.0
5	4200	3324-5004	571.3	3999	3324-4493	469.1	4402	3650-5004	593.0

Discussion

Previous inheritance studies hypothesized that the fused vein trait is governed by a single recessive gene, whose low and erratic inheritance results from gametophytic selection. The concept of gametophytic selection maintains that the male gametophyte genome imparts a functional ability, which is tested by the competitive processes of plant reproduction, resulting in the preferential transmission of superior alleles (Mulcahy, 1975; Zamir, 1983). Accordingly, any sporophytic trait, whose alleles differentially affect gametophytic function, will exhibit non-Mendelian inheritance and will be influenced by factors affecting reproductive competition. Together, the results of this chapter confirm the gametophytic selection hypothesis and evaluate its potentially adverse effects on seed crop production.

Both the pollen tube growth and pollen competition studies demonstrated the subvitality of the fused vein male gametophyte. Fused vein pollen tubes grew more slowly and were relatively less abundant in the style than normal pollen tubes. Fused vein pollen was less fertile, generating significantly fewer seed per fruit in all female genotypes. Its fecundity, unlike normal pollen, decreased with pollen load; seed yields for fused vein, F_1 and mixed pollinations decreased with load while normal yields remained stable. Relaxing reproductive competition also characteristically increased the recovery of fused vein segregants. These results indicate that the fused vein male gametophyte is less successful at traversing the reproductive path, executing fertilization and/or achieving normal seed development.

Windsor and coworkers (1987) have described two gametophytic selection mechanisms in *C. pepo*: prefertilization pollen competition and postfertilization fruit abortion. They showed that a high pollen load increases pollen competition, produces fruit with high seed set, and generates plants with superior vigor. In contrast, a low pollen load reduces competition, produces fruit with low seed set, and generates plants with inferior vigor. Low seed set also reduces the strength of a fruit's nutritional sink, leading to frequent fruit abortion. The two mechanisms act in tandem; fruit abortion arrests the development of offspring that do not arise from strong pollen competition. Our seed distribution study suggests that nutritional allocation also drives a third gametophytic selection mechanism within the fruit of *C. pepo*.

The inferior ovary of the *C. pepo* flower is syncarpous; the three to four carpels are cohesive and share a common central placenta that is continuous with the style and peduncle (Bailey and Swamy, 1951). The ovules within each locule are arranged linearly in two rows parallel to the flower axis. Each ovule is attached via its funiculus to the placenta and is nourished by vascular traces extending from the peduncle. To reach the ovules, pollen tubes grow through transmitting tissue in the style, into the ovary, and down the length of the placenta. Two gradients are inferred by this arrangement: a nutritional gradient with proximal ovules closest to the photosynthetic source, and a generative gradient with distal ovules closest to the stigmatic surface.

The seed distribution study revealed that, with low seed yields, there was preferential seed development within the fruit of NH2405.

Although flower dissections showed and even distribution of ovules, three quarters of the viable seed were produced in the proximal half of the fruit. Studies in *Zea mays* have shown that gametophytic advantage increases with time and distance. (Mulcahy, 1974; Johnson and Mulcahy, 1978; Ottaviano *et al*, 1983). The supposition is that nutritional proximity in *C. pepo* favors ovules fertilized by pollen tubes with the longest growth, thus selecting "superior" alleles. Observations made of many *C. pepo* fruit and fruit types over a fourteen year period support the possibility of ovule selection; with climatic or cultural stress, it is common to find low seed yields, uneven seed distributions, and partially developed seed. Limiting maternal investment to relatively few ovules is a common mechanism to enhance reproductive success among many plant species (Stephenson, 1981; Wyatt, 1981; Wiens, 1984; Stephenson and Windsor, 1986).

A difference in base level gametophyte vitality alone does not explain the observed variability of fused vein inheritance. Inheritance populations are typically generated with saturated pollen loads and strong pollen competition. One would expect not only low but constant fused vein frequencies if different levels of performance were the sole determinant of allele transmission. Presumably, the variable patterns of segregation resulted from fused vein and normal gametophytes also having different sensitivities to changing environmental conditions. The effects of the environment on the reproductive success of *C. pepo* and other cucurbits is well documented; it influences plant growth and vigor, flower production and anthesis, pollen quality and transmission, and set of fruit and seed

(Bushnell, 1920; Porter, 1933; Seaton and Kremer, 1938; Mann and Robinson, 1950; Matlop and Kelly, 1973, Sedgley and Buttrose, 1978; Iapichino and Loy, 1987; Hutton, 1988; Maestro and Alvarez, 1988;) In particular, Gay and colleagues (1987) have shown that *C. pepo* pollen germination and tube growth is sensitive to ageing and dehydration. Differential gametophyte performance to temperature stress has also been shown for *Cucumis melo* and several non cucurbit species (Herrero and Johnson, 1980; Zamir et al, 1981, 1982, 1987; Weinbaum *et al.*, 1984; Hutton, 1988; Maestro and Alvarez, 1988; Mulinix and Iezzoni, 1988; Rodriguez-Garay and Barrow, 1988). It is reasonable to assume that the difference between fused vein and normal gametophyte performance only widens with adverse condtions.

In summary, the low and erratic inheritance of the fused vein trait can be attributed to the combined action of poor male gametophyte performance, the reproductive biology of *C. pepo* and environmental variation. A high pollen load establishes strong male gametophyte competition which favors fertilization by normal gametes and reduces the inheritance of fused vein individuals. As pollen load decreases so does gametophyte competition increasing the likelihood of fused vein allele transmission. However, as pollen load decreases so does seed set which reduces the nutritional sink of a fruit, triggering the selective development of proximal seed and favoring normal allele transmission. If pollen loads and seed set are too low, fruit abortion occurs preventing either normal or fused vein inheritance. Changing environmental conditions variously effect the reproductive process, altering pollen load, gametophytic performance, and nutrient production.

The previous inheritance studies also suggested that differential female gametophyte production may affect fused vein inheritance. Low fused vein recovery among both reciprocal testcrosses implied a bias toward normal ovule production. This is supported by a discrepancy in fused vein recovery at the low pollen load in the pollen competition study. With low pollen load and presumably no male competition, the average fused vein recovery in testcross populations (Fused x F_1 and Fused x Mixed) approximated an expected Mendelian fifty percent, assuming a one gene model. In contrast, recovery in F_2 populations (F_1 x F_1 and F_1 x Mixed) was half the expected twenty-five percent, indicating preferential production of ovules from normal alleles in the F_1 female parent. There were, however, only eight F_2 populations at the low pollen load and fused vein recovery varied considerably, from 5.7 to 24.4 percent.

An association between gametophytic subvitality and reduced plant size and vigor has been demonstrated in several species: *Zea mays* (Mulcahy, 1974; Ottaviano *et al.*, 1980, 1983, 1988), *Dianthus chinensis* (Mulcahy and Mulcahy, 1975), *Petunia hybrida* (Mulcahy *et al.*, 1975) *Medicago sativa* (Mulinix and Iezzoni, 1988), and *C. pepo* (Windsor *et al*, 1987). Fortunately, the fused vein trait shows no such association. Although the plant growth study revealed that leaf initiation is slower in NH2405, its leaves are larger and provide a total leaf area equivalent to the normal line NH614. More importantly, there was no significant difference between the growth of fused vein and normal individuals in F_2 and backcross generations. Leaf initiation and size were independent of leaf morphology; both fused vein and normal individuals showed various

combinations of leaf size and number. F_2 and F_3 segregants from previous inheritance studies also showed no association between the trait and stem production; the plant habits of fused vein individuals ranged from single to multiple stems.

The gametophytic subvitality of the fused vein trait narrows its effectiveness as a rouging marker for hull-less seeded pumpkins. Successful hull-less seeded cultivars must not only be free of offtypes but must have acceptable plant and fruit characteristics and importantly a high seed yield. Although the fused vein trait does not adversely alter plant growth or structure, theoretically its inferior gametophytic fertility should reduce the potential seed yield of a fused vein cultivar relative to an isogenic normal cultivar. Nonetheless, the predominance of hybrid cultivars provides a solution.

Hybrid *C. pepo* cultivars are preferred because they provide earliness, adaptability, uniformity, productivity and varietal protection. Their production is accomplished by planting female and male parents in the same field, usually in a ratio of five rows to one. Staminate flower development is suppressed in the female line with several early applications of ethophon, an ethylene releasing chemical (Robinson *et al.*, 1970; Rudich *et al.*, 1969). Bees are employed to transfer pollen from the staminate flowers of the male line to the pistillate flowers of the female line. Only the first two or three fruit of the female parent are harvested because male flower suppression breaks down and inbred contamination of hybrid seed lots can occur.

A fused vein by normal hull-less seeded hybrid permits partial exploitation of the trait's morphological uniqueness while avoiding its adverse effect on yield. The distinctive juvenile morphology of the fused vein trait facilitates increase and maintenance of the female parent. Hulled/normal offtypes are easily identified and removed from parent and hybrid production fields before contamination can spread. Although increase of the normal male parent requires more stringent and costly control, the smaller quantity of seed required for hybrid production reduces the frequency of male inbred increases and spreads the added cost over several hybrid productions. As a heterozygote, the hybrid has a normal leaf phenotype and produces ample normal pollen to compensate for the reduced fertility its fused vein pollen. As an added advantage, its normal phenotype enables easy detection of fused vein inbred contamination in hybrid seed lots.

CHAPTER VI

CONCLUSIONS

Hull-less seeded pumpkin cultivars are prone to repeated contamination by outcrossing with morphologically similar hulled cultivars. Detection and removal of offtypes is encumbered by the similarity of juvenile plant morphology, and the maternal expression of the hull-less trait. Use of a rouging marker can facilitate the maintenance and production of hull-less seeded cultivars, fostering their expanded use as a seed and oil crop. This dissertation examined whether the fused vein trait can serve as a suitable rouging marker for hull-less cultivars. It specifically characterized the morphology and anatomy of the trait, determined its mode of inheritance, and evaluated its effect on gametophytic fecundity and sporophytic growth.

Morphological examination of the fused vein inbred NH2405, showed that the trait is characterized by the partial fusion of the lateral leaf veins to the main central vein. Fusion begins at the distal point of the petiole and extends 10 to 15 centimeters into the leaf blade. Consequently, the dorsal leaf surface appears puckered. The extent of fusion along the central vein is less on early leaves and becomes pronounced by the tenth leaf stage. The trait is expressed beginning at the fourth leaf stage, prior to flowering, and then throughout vegetative growth. Although phenotypic expression is uniform within a line, it varies between lines. Inspection of the moderately fused vein line, NH7210, and fused

vein segregants in F_2 and F_3 populations revealed that the onset of fused leaf production and the extent of vein fusion vary.

Anatomical examination of NH2405 revealed that vein fusion results from a delay in vein development and separation rather than the adhesion of completed structures. Comparison with normal leaves uncovered different vascular patterns in the transition zone between petiole and leaf blade. Normal leaves have one cycle of enlargement, coalescence and dispersal, while fused vein leaves have two. A parallel pattern of expression between vein fusion and leaf lobing and a petiole-like distribution of vascular bundles in the leaf transition zone indicated that the trait interferes with marginal meristem activity, blurring the developmental boundary between the petiole and leaf blade.

Inheritance data from crosses between NH2405 and the normal lines NH614 and NHBP10 did not fit expected Mendelian ratios. Fused vein recovery was low and erratic in segregating populations. It was hypothesized that the fused vein trait is governed by a single recessive gene that experiences gametophytic selection. In coupling phase, the fused vein character also skewed the inheritance of the hull-less seeded trait, indicating an association and possible lingage. Assuming single gene inheritance, linkage which was calculated to be 29.2 map units.

The gametophytic selection hypothesis was tested and confirmed by comparing both *in situ* pollen tube growth and the relative fecundities of fused vein and normal pollen. Fuse vein gametophytes were less capable of traversing the reproductive path and effecting fertilization. Their pollen tubes were less numerous in stylar tissue and grew more slowly.

Fused vein pollen produced significantly few seed per fruit across all female genotypes and was also more sensitive to pollen load reduction. As expected for a gametophytic subvital, relaxation of reproductive competition also resulted in a corresponding increase in fused vein inheritance in segregating populations.

Comparison of leaf initiation and total leaf area for NH2405, NH614, and their F_1, F_2 and testcross generations showed that plant growth is independent of leaf phenotype. Although NH2405 produced fewer leaves than NH614 they were larger and resulted in the same total photosynthetic area. The association of fewer, larger leaves with the fused vein genotype also disappeared in the segregating populations, where both normal and fused vein plants exhibited similar combinations of leaf number and size.

The rarity, distinct morphology, and recessive inheritance of the fused vein trait make it a suitable rouging marker for hull-less cultivars. Its discovery in a hull-less line was fortuitous. Its association with the hull-less gene will facilitate its incorporation into other hull-less lines and will help to limit its use to hull-less material. The trait's gametophytic subvitality, however, narrows its usefulness. Although there is no associated effect on plant growth, it does reduce sporophytic seed yield. Fused vein by normal hybrid cultivars will be required to exploit the trait's unique morphology and to avoid its detrimental effect on yield.

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