Fused Vein Trait in *Cucurbita pepo* L. Associated with Subvitality of the Male Gametophyte

R. Bruce Carle and J. Brent Loy
Department of Plant Biology, University of New Hampshire, Durham, NH 03824

Abstract. Two experiments were conducted to test and delineate gametophytic subvitality of the fused vein trait in *Cucurbita pepo*. Gametophytic subvitality was verified by comparing pollen tube growth for fused vein and normal pollen in situ. Microscopic examination of partitioned, co-pollinated distillate flowers revealed inferior fused vein gametophyte performance. Normal pollen tubes grew faster and were significantly more abundant in the lower portion of the style. The consequences of gametophytic subvitality on seed yield and inheritance were shown by manipulating the severity of pollen competition. Fused vein, normal and F lines were pollinated with fused vein, normal, F and a 50:50 pollen mix at three different pollen loads. Fused vein pollen generated significantly fewer seed per fruit in all female genotypes. As a constituent in F, or mixed pollen, it produced significant seed yield reductions at the low pollen load. In F, and testcross populations, a reduction in pollen load and therefore pollen competition significantly increased the number of fused vein individuals in segregating populations.

A new leaf mutant, fused vein (fv), in *Cucurbita pepo* has potential as a roguing marker for hull-less seeded pumpkins. The fused vein (FV) trait is expressed before flowering and imparts a distinctive, readily visible, leaf morphology (Carle and Loy, 1996a). The trait is not present among current cultivars and is likely governed by a single recessive gene (Carle and Loy, 1996b). Combined, these attributes allow early and easy outcross detection from a broad range of potential contaminants. Inheritance studies, however, suggest that the trait imparts a gametophytic subvitality that could limit its usefulness.

Gametophytes are not 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 ovules (Zamir, 1983). Selection of gametophytes changes allele frequencies in the sporophyte generation. Sporophytic traits that enhance gametophyte performance increase at the expense of those that diminish performance. 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 FV trait does not alter plant size or vigor (Carle and Loy, 1996a). The purposes of the following studies were to confirm the FV trait’s gametophytic subvitality and to determine its impact on seed production.

Materials and Methods

*In situ pollen tube growth study.* The FV inbred line NH2405, the normal (N) inbred line NH614 (J.B. Loy, Univ. of New Hampshire (UNH), Durham), and their F hybrid were used to examine FV and N pollen tube growth. Eight plants of each line were raised in the UNH greenhouse during Spring 1992. All plants received the same cultural treatments: 9.5-liter pots, Promix soilless medium, periodic feeding with water-soluble fertilizer (Peters 20–20–20), and standard pest control.

Beginning 15 Mar. and continuing for 2 weeks, controlled co-

Received for publication 23 Jan, 1995. Accepted for publication 10 July 1995. Scientific contribution no. 1892 from the New Hampshire Agricultural Experiment Station, Durham. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.
tions. The second plot was planted 30 May and consisted of 18 m
rows, one for each line, at the same plant and row spacing as above.

From 14 July to 7 Aug., hand pollinations were made between
0700 and 1000 hr on all three lines. Female and male flowers were
identified a day before anthesis and protected from natural pollination
by tying their corollas shut with twist ties. Following anthesis,
female flowers were designated at random for treatment and male
flowers were collected to provide four pollen sources: FV, N, F,
and a 50:50 mixture (M) of FV and N 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
Windsor et al. (1987). For saturated pollinations, the pollen from
well-dehisced anthers of two 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 cotton swab cut in half was
used to collect and apply pollen. A thin and uniform pollen layer
on the bottom and lower 2 mm of the cut end formed a single
application. Counts of 35 applications onto glass slides yielded on
average 233 (SD = 3.5) pollen grains per application. Two applica-
tions per stigma were used for the low pollen load and four
applications were used for the medium load. The low level was
selected to minimize pollen competition. The number of pollen
gains approximated the highest seed yields obtained from pollina-
tions in a previous inheritance study. Medium and saturated loads
were chosen to respectively double and maximize competition.
The 50:50 mixture treatments were accomplished by dividing
applications equally between FV and N pollen.

Each day the 36 different treatment pollinations were made in
groups, as complete as possible, determined by the availability of
female and male flowers. Abortion of pollinated female flowers,
particularly for low and medium pollen loads, resulted in incom-
plete and unequal numbers of fruit for pollination groups and
precluded their comparison as individual replicates. By the end of
the pollination period, a minimum of 15 fruit 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 devel-
oping fruit were removed to limit fruit set to two to three fruit per
plant.

Growth, flowering, and fruit production were the same in both
plots. Fruit were harvested in mid-September after a minimum of
40 days maturation. Each fruit was deseeded individually, and its
seeds cleaned, dried, and counted. Seed counts from diseased fruit
were included in analysis only if the total number of normally
developed seeds could be determined. Analyses of variance for a
random design with unequal subsamples were used to test for
significant treatment effects. Fruit from the second plot did not
constitute a complete group of treatments; plot difference was
assumed to be negligible, and seed counts were combined from
both plots for analysis. Mean comparisons for pollen source,
pollen source within pollen load, and pollen load within pollen
source were performed using least significant difference (LSD).

Pollinations that generated F1 and testcross populations were
examined for FV recovery by pollen load. In three cycles of
growouts from October 1991 to April 1992,30 seeds derived from
each pollination (F1 × F2; 3 low, 6 medium, 10 saturated; F1 × M ;
5 low, 5 medium, 3 saturated; FV × F2; 2 low, 4 medium, 1
saturated; and FV × M; 3 low, 8 medium, 3 saturate) were sown in
15-cm (1.9-liter) pots in the greenhouse. Resulting plants were
grown until the tenth leaf stage and scored for leaf phenotype.
Progeny were designated FV if they exhibited continual FV leaf
production by the tenth leaf. All other plants were considered N.

Chi-square analyses were performed using one and two gene
models for determining expected ratios. The frequencies of FV
progeny were determined and analyzed using the Kruskal-Wallis
Test and Kolmogorov-Smirnov two sample comparisons (Steel
and Torrie, 1980).

Results

In situ pollen tube growth. 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 whatsoever. It was judged abrant
and four missing data points were estimated following Steel
and Torrie (1980). Regardless of genotype, pollen tube number
increased over time and diminished with distance traveled. In two
different five-hour pollinations, FV and N pollen tubes failed to
reach the base of the style. In both instances, however, N tubes
outgrew FV tubes. Pairwise t test comparisons revealed that with
time, there was a significant genotype effect on pollen tube growth
in co-pollinated treatments (Table 1). The trend of N pollen tubes
being more abundant than FV tubes became significant at 8 h in the
ovary end of the style (Fig. 1).

Table 1. Pairwise t test comparisons of pollen tube growth by style
position and by pollen genotype at 5- and 8-h periods.

| Comparison                        | 5 h    | 8 h    |
|-----------------------------------|--------|--------|
| Fused vein pollen                 | 4.17***| 5.30***|
| Normal pollen                     | 4.17***| 0.42***|
| Fused vein vs. normal pollen      |        |        |
| Stigma end                        | 1.37 ns | 1.53 ns |
| Ovary end                         | 1.10 ns | 4.25***|

**,** ***Nonsignificant or significant at P ≤ 0.010.

Effect of pollen source and load on seed yield. Pairwise LSD
comparisons of open pollinated yields revealed significant differ-
ces (P= 0.05) among the three female genotypes (Table 2). The
FV line yielded, on average, fewer seed per fruit then the N line,
while the F1 exhibited heterosis, out-yielding either parent. These
inherent yield differences and unequal treatment replications
prompted separate analyses for each female line and each cross.

Random linear models were constructed to test the effects of
pollen source and pollen load on seed yield. Pollen source had a
significant influence on yield for both the N (P = 0.05; F = 4.29; df
= 3, 23) and F1 lines (P= 0.05; F = 5.14; df = 3, 37) and was near
significant for the FV line (P = 0.10; F = 2.24; df = 3, 40). In
contrast, pollen load did not significantly alter yield when indi-
vidual crosses were combined. Pollen load was, however, highly
significant for two individual crosses, FV × FV (P = 0.01; F =
11.85; df=2,9) and N × M (P = 0.01, F = 15.81, df = 2,7), and near
significant for three other crosses, N × F1 (P= 0.10; F = 3.23; df
= 2, 15), F1 × F (P = 0.10; F = 3.24; df = 2,32), and F1 × M (P = 0.10;
F = 3.74; df = 2, 11).

LSD comparison of pollen sources with combined loads, as
suggested by the analysis of variance, revealed that FV pollen
significantly lowered seed yield (Table 3). The combined load
means also revealed the trend that M pollen produced lower
average yields than FV pollen, which in turn produced lower yields
than N pollen. The individual effects of pollen source by load on
seed yield were also examined by pairwise LSD comparisons. FV
pollen produced significantly (P = 0.05) lower average seed yields

J. AMER. SOC. HORT. SCI. 121(1): 18-22.1996.
Fig. 1. Comparison of mean pollen tube growth by genotype, time, and style section. F female pistils were partitioned into separate halves, one half pollinated with fused vein pollen the other pollinated with normal pollen. Pollen tube growth was scored for stigma and ovary ends of the style: 0 for no tubes, 1 = 1–20, 2 = 21–40, 3 = 41–60, 4 = 61–80, and 5 = 81 or more tubes. Thin bars represent SF for growth score means.

Table 2. Mean seed yield for open-pollinated fruit.

| Fruit type | Sample no. | Mean | Range  | Standard deviation |
|------------|------------|------|--------|--------------------|
| Fused vein | 18         | 330 a | 121–432| 87.7               |
| Normal     | 17         | 449 b | 278–638| 85.5               |
| F, hybrid  | 15         | 535 c | 264–691| 128.2              |

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

Table 3. Mean seed yield by pollen source with combined pollen loads for fused vein, normal, and F1 fruit.

| Female parent | Pollen source | Fused vein | Normal | F1 hybrid |
|---------------|---------------|------------|--------|-----------|
|               | Fused vein    | 169 a      | 155 a  | 273 a     |
|               | 50:50 mix     | 233 b      | 221 b  | 356 b     |
|               | F, hybrid     | 240 b      | 271 bc | 428 c     |
|               | Normal        | 271 b      | 312 c  | 416 bc    |
|               | Open pollinated | 330 b   | 449 d  | 535 d     |

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

Fig. 2. Effect of pollen load (A–C) and pollen source (D–F) on average seed yield for fused vein, normal, and F1 fruit. The low, medium, and saturated pollen loads were about 460, 920, and 5000+ grains, respectively. Different letters within a bar cluster denote significant differences between means. Pairwise LSD tests (P < 0.05) were used due to the unequal number of samples for each treatment.

Than N pollen for six of the nine sets of load by source comparisons (Fig. 2 A–C). In general, N, F1, and M pollen were not significantly different from each other. N pollen, however, produced the highest average yields, particularly at the low pollen load. Although the analyses of variance indicated no significant pollen load effect, LSD comparisons showed decreased seed yields at the low pollen load, relative to the saturated load, for a majority of crosses involving FV, F1, and M pollen (Fig. 2 D–F). In contrast, crosses with N pollen showed no significant decreases, suggesting a weakness of the FV gametophyte.

Pollen competition and FV recovery. The frequencies of FV progeny obtained in F1 and testcross populations varied considerably. As in previous inheritance studies (Carle and Loy, 1996b), chi square analysis showed fit to both single and double recessive gene models for both F1 (Fig. 3) and testcross populations (Fig. 4). However, fit was also associated with pollen load. Only one single gene fit occurred at the saturated pollen loads. Single gene fit predominated at the low load for testcross populations and at the medium load for F1 populations. Failure to recover FV individuals occurred only at the saturated load for F1 populations generated with F1 pollen.

Because the distribution of F1FV frequencies obtained with saturated F1 pollen was skewed, data were analyzed nonparametrically. Kruskal-Wallis analysis revealed a significant pollen load effect, but no significant difference between pollen sources for both the F1 and
In previous inheritance studies, Carle and Loy (1996b) hypothesized that the FV trait is governed by a single recessive gene, whose low and erratic inheritance results from gametophytic subvitality. The concept of gametophytic selection maintains that whose alleles differentially affect gametophytic function, will exhibit distorted patterns of Mendelian inheritance and will be influenced by factors affecting reproductive competition.

Both the pollen tube growth and pollen competition studies demonstrated the subvitality of the FV male gametophyte. FV pollen tubes grew more slowly and were relatively less abundant in the style than N pollen tubes. FV pollen generated significantly fewer seeds per fruit than N-pollen in all female genotypes. Its fecundity, unlike N pollen, decreased with pollen load. Seed yields for FV, F, and M pollinations decreased with load while N yields remained stable. Relaxing reproductive competition also characteristically increased the recovery of FV segregants. These results indicate that the FV male gametophyte is less successful at traversing the reproductive path, achieving fertilization, and realizing normal seed development.

Presumably, the variability of FV inheritance and seed yields results not only from a difference in base level gametophytic ability but from differential 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 flower production and anthesis (Seaton and Kremer, 1938; Sedgley and Buttrose, 1978), pollen quality and transmission (Hutton, 1988; Iapichino and Loy, 1987; Maestro and Alvarez, 1988; Matlop and Kelly, 1973; Sedgley and Buttrose, 1978), and set of fruit and seed (Bushnell, 1920; Mann and Robinson, 1950; Porter, 1933). 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 (Hutton, 1988; Maestro and Alvarez, 1988). We assume that the difference between FV and N gametophyte performance only widens with adverse conditions.

The gametophytic subvitality of the FV trait narrows its effectiveness as a roguing marker for hull-less seeded pumpkins. Successful hull-less seeded cultivars must not only be free of off-types, but must have acceptable plant and fruit characteristics and, importantly, a high seed yield. Although the FV trait does not adversely alter plant growth or structure (Carle and Loy, 1996a), theoretically its inferior gametophytic fertility should reduce the potential seed yield of a FV cultivar relative to an isogenic N cultivar. Nonetheless, the predominance of hybrid cultivars provides a solution. A FV by N 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 FV trait facilitates increase and maintenance of the female parent. Hulled, normal off-types are easily identified and removed from parent and hybrid production fields before contamination can spread. Although increase of the N male parent requires more stringent and costly control, the smaller quantity of seed of the

**Discussion**

In previous inheritance studies, Carle and Loy (1996b) hypothesized that the FV trait is governed by a single recessive gene, whose low and erratic inheritance results from gametophytic subvitality. The concept of gametophytic selection maintains that the male gametophyte genome imparts a functional ability, which

---

**Table 4. Mean fused vein frequencies for F_1 and backcross populations by pollen load and pollen source.**

| Pollen Source | F_1 | Mixed | Combined | F_2 | Mixed | Combined |
|---------------|-----|-------|----------|-----|-------|----------|
| Low           | 0.118 a  | 0.132 ab | 0.128 a  | 0.576 a  | 0.456 a  | 0.503 a  |
| Medium        | 0.127 a  | 0.178 a  | 0.151 a  | 0.415 b  | 0.349 ab | 0.372 b  |

Different letters in the same column denote significantly different means, Kolmogorov-Smirnov ranked two sample tests (P< 0.05).

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 distorted patterns of Mendelian inheritance and will be influenced by factors affecting reproductive competition.

The effects of the environment on the reproductive success of C. pepo and other cucurbits is well documented; it influences flower production and anthesis (Seaton and Kremer, 1938; Sedgley and Buttrose, 1978), pollen quality and transmission (Hutton, 1988; Iapichino and Loy, 1987; Maestro and Alvarez, 1988; Matlop and Kelly, 1973; Sedgley and Buttrose, 1978), and set of fruit and seed (Bushnell, 1920; Mann and Robinson, 1950; Porter, 1933). 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 (Hutton, 1988; Maestro and Alvarez, 1988). We assume that the difference between FV and N gametophyte performance only widens with adverse conditions.

The gametophytic subvitality of the FV trait narrows its effectiveness as a roguing marker for hull-less seeded pumpkins. Successful hull-less seeded cultivars must not only be free of off-types, but must have acceptable plant and fruit characteristics and, importantly, a high seed yield. Although the FV trait does not adversely alter plant growth or structure (Carle and Loy, 1996a), theoretically its inferior gametophytic fertility should reduce the potential seed yield of a FV cultivar relative to an isogenic N cultivar. Nonetheless, the predominance of hybrid cultivars provides a solution. A FV by N 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 FV trait facilitates increase and maintenance of the female parent. Hulled, normal off-types are easily identified and removed from parent and hybrid production fields before contamination can spread. Although increase of the N male parent requires more stringent and costly control, the smaller quantity of seed of the
male parent 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 N leaf phenotype and produces ample N pollen to compensate for the reduced fertility of its FV pollen. As an added advantage, its N phenotype enables easy detection of FV inbred contamination in hybrid seed lots.

**Literature Cited**

Bushnell, J.W. 1920. The fertility and fruiting habit of the cucurbita. Proc. Amer. Soc. Hort. Sci. 17:47–51.

Carle, R.B. and J.B. Loy. 1996a. Morphology and anatomy of the fused vein trait in *Cucurbita pepo* L. J. Amer. Soc. Hort. Sci. 121:6–12.

Carle, R.B. and J.B. Loy. 1996b. Genetic analysis of the fused vein trait in *Cucurbita pepo* L. J. Amer. Soc. Hort. Sci. 121:13–17.

Gay, G., C. Kerhoas and C. Dumas. 1987. Quality of stress-sensitive *Cucurbita pepo* L. pollen. Planta 171:82–87.

Hutton, M.G. 1988. Genetics and physiology of cold tolerance in muskmelon *Cucumis melo* L. PhD diss., Univ. of New Hampshire

Iapichino, G.F. and J.B. Loy. 1987. High temperature stress affects pollen viability in bottle gourd. J. Amer. Soc. Hort. Sci. 112:372–374.

Maestro, C.A. and J. Alvarez. 1988. The effects of temperature on pollination and pollen tube growth in muskmelon (*Cucumis melo* L.). Scientia Hort. 36:173-181.

Mann, L.K. and Robinson, J. 1950. Fertilization, seed development, and fruit growth as related to fruit set in the cantaloupe (*Cucumis melo* L.). Amer. J. Bet. 37:685–697.

Matlop, A.N. and W.C. Kelly. 1973. The effect of high temperature on pollen tube growth of snake-melon and cucumber. J. Amer. Soc. Hort. Sci. 98:296–300.

Mulcahy, D.L. 1974. Correlation between speed of pollen tube growth and seedling height in *Zea mays* L. Nature 249:491493.

Mulcahy, D.L. 1975. The biological significance of gamete competition, p. 1–3. In: D.L. Mulcahy (ed.). Gamete competition in plants and animals. North Holland Publ. Co., Amsterdam.

Mulcahy, D.L. and G.B. Mulcahy. 1975. The influence of gametophytic competition on sporophytic quality in *Dianthus chinensis*. Theoret. Appl. Genet. 46:277–280.

Mulcahy, D.L., G.B. Mulcahy, and E. Ottaviano. 1975. Sporophytic expression of gametophytic competition in *Petunia hybrids*, p. 227–232. In: D.L. Mulcahy (ed.). Gamete competition in plants and animals. North Holland Publishing Co., Amsterdam.

Ottaviano, E., D. Petroni, and M.E. Pe’. 1988. Gametophytic expression of genes controlling endosperm development in maize. Theoret. Appl. Genet. 75:252–258.

Porter, D.R. 1933. Watermelon breeding. Hilgardia 7:585–624.

Seaton, H.L. and J.C. Kremer. 1938. The influence of climatological factors on anthesis and anther dehiscence in the cultivated cucurbits. Proc. Amer. Soc. Hort. Sci. 36:627–631.

Sedgley, M. and M.S. Butrose. 1978. Some effects of light intensity, daylength, and temperature on flowering and pollen tube growth in the watermelon (*Citrullus lanatus*). Ann. Bot. 42:609–616.

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

Windsor, J.A, L.E. Davis, and A.G. Stephenson. 1987. The relationship between pollen load and fruit maturation and the effect of pollen load on offspring vigor in *Cucurbita pepo*. Amer. Nat. 129:643-656.

Zamir, D. 1983. Pollen gene expression and selection: applications to plant breeding, p. 3 13–330. In: S.D. Tanksley and T.J. Orton (eds.). Isozymes in plant genetics and breeding, part A. Elsevier, New York.