Expression of the Tendrilled Acacia (*tac*) Trait in the Afila-Tendrilled Acacia (*afaf tactac*) Pea Leaf during Plant Development

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The afila (*af*) and tendrilled-acacia (*tac*) alleles modify the normal pea leaf. The *af* allele replaces leaflets with tendrils, and the *tac* allele restores small leaflets to the terminal portion of the leaf. Lower leaves of the resultant *afaf tactac* foliage type are characterized by large tendrils that terminate in small laminae, while the upper leaves exhibit pairs of opposite leaflets similar to those found in a normal pea leaf. A series of near-isogenic normal (*AfAf TacTac*), tendrilled acacia (*AfAf tactac*), and afila-tendrilled acacia (*afaf tactac*) foliage types in a range of genetic backgrounds was evaluated for the relative percentage of either normal leaflets or *tac* leaflets to the total pea leaf area and weight at successive node positions. The objective was to evaluate and quantify shifts in leaf morphology associated with these three foliage types. Foliage types were grown in a drill-plot experiment over 2 years in two locations, a wire-trellis experiment over 2 years in one location, and a greenhouse experiment. Results show that the *tac* allele in *afaf* plants causes a return to the *AfAf,* or normal foliage type, as measured by the percentage of the leaf composed of leaflets. The percentage of the total leaf area due to *tac* leaflets in field-grown *afaf tactac* plants changed from 27% in nodes below the first pod-bearing node to over 80% in nodes above the first pod-bearing node. This morphological shift was evident in all three experiments. Similar results were observed for percentages of total leaf weight.

The garden pea (*Pisum sativum* L.) possesses a vast array of genes that alter foliage morphology. Some of these genes condition morphological changes in the leaf that may improve the standard foliage type. Homeotic mutants afila (*af*) and tendrilled-acacia (*tac*) are responsible for dramatic structural changes in the pea leaf. The *af* allele replaces leaflets with tendrils (Kujala 1953), and *tac* restores small leaflets to tendrils of *af* plants (Sharma 1972). Lower leaves of the resulting *afaf tactac* leaf phenotype are characterized by large tendrils that terminate in small leaflets, while the upper leaves exhibit pairs of opposite leaflets similar to those found in a normal pea leaf. The *tac* allele in combination with *Af* results in a phenotype similar to normal (*AfAf TacTac*), except that the leaves contain a terminal *tac* leaflet in place of the terminal tendril. The agronomic performance of the *afaf tactac* foliage type has been investigated (Goldman and Gritton 1991; Goldman et al. 1991). Morphological changes associated with leaf development in plants carrying both *af* and *tac* have not yet been examined.

Morphological changes that were seemingly associated with development in the mutant pea leaf were first described when the *afaf tltl tactac* foliage type was studied by Marx (1989). The acacia (*tl*) allele is responsible for converting tendrils to leaflets in the pea leaf. Marx (1989) reported a developmental change in *afaf tltl tactac* that was not apparent in *afaf tltl TacTac.* Although leaves containing both *af* and *tac* are typically of a branched three-dimensional nature, Marx noticed that later-formed leaves exhibited the essentially planar surface of normal (*AfAf*) pea leaves when *tac* was added to the *afaf tltl* genotype. This observation suggests that a developmentally-associated change in leaf morphology may be due to "cancellation" of the typical effects of *af.*

Major morphological changes associated with development have been associated with single gene changes in other plant species, such as *Cg* in maize (Ritchings and Tracy, 1989). A series of homeotic genes in *Antirrhinum majus* that control flower development has been reported by Schwarz-Sommer et al. (1990). Homeotic

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mutants that affect organization of floral organs in *Arabidopsis thaliana* have been well documented by Bowman et al. (1989). Poethig (1990) reviewed the regulation of shoot morphogenesis by various mutants and concluded that phase changes are due to distinct but overlapping developmental programs.

During studies designed to evaluate the agronomic performance of the *afaf tactac* type in pea (Goldman and Gritton 1991; Goldman et al. 1991), a morphological change similar to the type described by Marx was noticed. Several experiments were devised to quantify and further examine this change in expression of the *tac* trait as it relates to morphology of the *AfAf tactac* and *afaf tactac* pea foliage types.

Materials and Methods

Genetic Material

Normal foliage (*AfAf TacTac*) and afila-tendrilled acacia foliage (*afaf tactac*) near-isogenic lines in three genetic backgrounds:—Alsweet 4683, Frontier, and Dark Skin Perfection—were used in Experiment 1. Experiments 2 and 3 made use of a single *afaf tactac* line in the Novella background, a set of normal foliage and tendrilled acacia foliage (*AfAf tactac*) near-isogenic lines in the Badger background, and *afaf tactac* lines in the Alsweet 4683, Frontier, and Dark Skin Perfection backgrounds. The *AfAf tactac* type has pairs of "normal" leaflets and a terminal *tac* leaflet. All of these genetic backgrounds are of the type used for canning and freezing. Plant material containing the *tac* allele used in these studies was obtained from B. Sharma (Division of Genetics, Indian Agricultural Research Institute, New Delhi, India) and incorporated via the backcross procedure. The sixth backcross was made in fall 1986, and the BC_6F_1 plants were grown in the greenhouse in spring 1987. All near-isolines were BC_6F_6-derived at the beginning of the study.

Experiment 1

We planted four replications of a split-plot treatment arrangement of a randomized complete block design in 1.07 × 1.52 m seven-row simulated drill-plots with a row spacing of 18 cm. Plots were hand sown to a density of 1.34×10^6 plants/ha. The experiment was conducted in two environments: the Arlington Agricultural Research Station, Arlington, Wisconsin, in 1989 on Plano silt loam soil (Typic Arguidolls), and the West Madison Agricultural Research Station, Madison, Wisconsin, in 1989 on Plano silt loam soil (Typic Arguidolls). In each experiment, we assigned genetic background to the whole plot and foliage type to the subplot.

Planting dates were 27 April at Arlington in 1989, and 3 May at West Madison in 1989. We treated seeds with the fungicide Captan (N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide) before planting and sowed them by hand in 7.6-cm-deep furrows. Seeds were spaced 2.5 cm apart in the row. We incorporated trifluralin (α-α-α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) before planting at a rate of 0.17 L ha^{-1} in all trials for weed control. Supplemental weeding was by hand.

A 600-cm^2 bordered section of the plot was removed at the green pea harvest stage (approximately 100 tenderometer, the optimal harvest stage for commercial pea processing). We simultaneously removed all three foliage types in a given genetic background at this sampling date. The entire 600-cm^2 section was transported to Madison where we randomly chose a three-plant sample. The decision to sample three plants was based on sample size estimates reported by Gritton and Chi (1972). We placed each sample in an airtight plastic bag and then in a refrigerator, and we analyzed samples within 48 h. Since sufficient material for tenderometer evaluation was not available for the final sampling date in this study, sampling decisions were based on tenderometer readings of similar cultivars in an adjacent trial.

The three-plant sample was separated into the foliage components stipule, leaflet, tendril plus petiole, and *tac* leaflet. Foliage area of the tendril plus petiole, leaflet, and *tac* leaflet components at each node above the first nonsenescent node were measured with a Li-cor 3100 leaf area meter (Li-cor, Lincoln, Nebraska). Node counts began with the first scale leaf. The area of tendril plus petiole was corrected by $\pi/2$ (Harvey and Goodwin 1978) to account for its cylindrical shape. We measured tendrils and leaflets for *AfAf TacTac* plants, and tendrils and *tac* leaflets on *afaf tactac* plants. The percentage of total leaf area due to leaflets (in *AfAf TacTac* plants) and *tac* leaflets (in *afaf tactac* plants) was calculated for each node. Stipule area was not included in this calculation. The average lowest pod-bearing node from each sample was also recorded.

Experiment 2

Experiment 2 was conducted at the Arlington Agricultural Research Station, Arlington, Wisconsin, during 1990. The study was planted on May 2 along one side of a wire trellis in plots 30 cm in length. Twelve seeds were sown in each plot. The design of the experiment was a randomized complete block with four replications. The wire trellis environment is commonly used by pea breeders as a way to evaluate germplasm (Gritton 1980). Trellis culture is characterized by little interplant competition and the absence of lodging, due to the twining of tendrils on wires. Trifluralin (α-α-α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) was incorporated before planting at a rate of 0.017 L ha^{-1} to control weeds. Supplemental weeding was done by hand. We harvested all plots on July 12 (approximately 21 days after 50% bloom of early-maturing lines), placed samples in airtight plastic bags, and transported them to the laboratory in Madison for growth analysis. Three plants were chosen randomly from the sample. We separated plants for the foliage components tendril plus petiole, leaflet, and *tac* leaflet. Foliage components were then placed in paper bags, dried at 56°C for 48 h, and weighed. Data were expressed as the percentage of leaf weight due to leaflet (in the case of *AfAf TacTac* and *AfAf tactac* plants) and *tac* leaflet (in *afaf tactac* plants).

Experiment 3

Experiment 3 was conducted in the greenhouse during 1990, using a randomized complete block design with three replicates. Five seeds of each genotype were sown in each 20-cm pot on January 5. We maintained an 18-h daylength throughout the study, and the average daily temperature was 21°C. Soil fertility was maintained by weekly applications of a water-soluble 20-20-20 fertilizer. We randomly selected three plants in each pot for growth analysis on February 23. We separated plants into the foliage components tendril plus petiole, leaflet, and *tac* leaflet; plant components were then placed in paper bags and dried for 48 h at 56°C. Foliage components were weighed, and data were expressed as the percentage of the leaf due to *tac* leaflets (in the case of the four *afaf tactac* lines and the terminal *tac* leaflet in the single *AfAf tactac* line) or the percentage of the leaf due to normal leaflets (in the case of the *AfAf TacTac* line).

Statistical Analysis

Percentage of total leaf weight or total leaf area due to *tac* leaflets in *afaf tactac* plants, normal leaflets in *AfAf TacTac* plants, and *tac* leaflets in *AfAf tactac* plants was regressed on mean node number in each

experiment. All regression analyses were performed using the REG procedure of SAS (Statistical Analysis System, Cary, North Carolina). Beginning with a linear model, we performed regressions of higher order polynomials and determined the best-fitting equation for each line by *t* test. All lines in these experiments were fit by linear or quadratic models. The general lack of significant environment × percentage of total leaf weight or area interaction permitted regression analysis to be performed on combined data (1989 and 1990) in Experiment 1. Since identical trends were observed among genetic backgrounds for the percentage of total leaf area or weight due to leaflets (for *AfAf TacTac* genetic backgrounds) or *tac* leaflets (for *afaf tactac* genetic backgrounds), data in Experiment 1 were averaged over genetic backgrounds. Data in Experiments 2 and 3 were also averaged over genetic backgrounds with the exception of the *AfAf tactac* category, for which only one background was used.

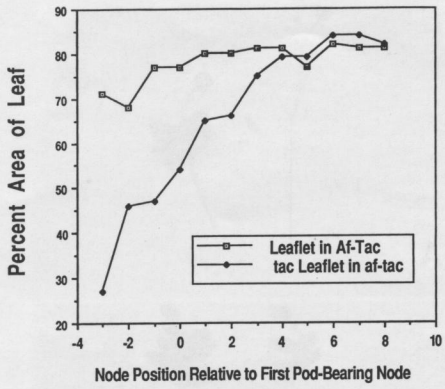


Figure 1. Percentage of pea leaf due to *tac* leaflets in *afaf tactac* plants or normal leaflets in *AfAf TacTac* plants relative to the lowest pod-bearing node. The equation $y = 0.754 + 0.007$ Node describes the response of normal leaflets, and the equation $y = 0.056 + 0.072$ Node $- 0.005$ Node^2 describes the response of *tac* leaflets. The R^2 values associated with these equations were 0.33 and 0.98, respectively.

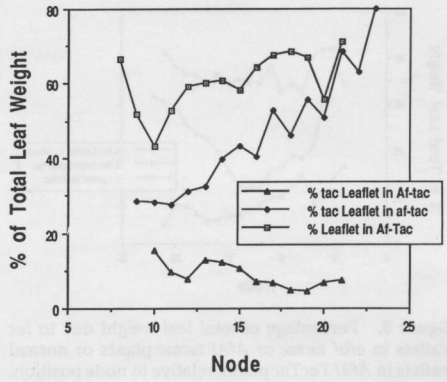


Figure 2. Percentage of total leaf weight due to *tac* leaflets in *afaf tactac* or *AfAf tactac* plants or normal leaflets in *AfAf TacTac* plants relative to node position. The equation $y = 45.322 + 1.045$ Node describes the response of normal leaflets. The equation $y = 32.128 - 2.058$ Node $+ 0.170$ Node^2 describes the response of *tac* leaflets in *afaf tactac* plants. The equation $y = 147.705 - 15.428$ Node $+ 0.523$ Node^2 describes the response of *tac* leaflets in *AfAf tactac* plants. The R^2 values associated with these lines were 0.31, 0.93, and 0.85, respectively.

Results and Discussion

Experiment 1

The response curve of the percentage of total leaf area due to *tac* leaflets in *afaf tactac* plants (averaged over genetic backgrounds) was fit by a quadratic model (Figure 1). The percentage of leaf area due to normal leaflets in *AfAf TacTac* plants was explained by a linear model. These equations are given in the caption below Figure 1. The response of these foliage types indicates that leaflet expression in *AfAf TacTac* plants remains relatively constant throughout ontogeny, whereas the expression of *tac* leaflets in *afaf tactac* plants increases as development progresses. At three nodes below the first pod-bearing node, the *AfAf TacTac* leaf is made up of 71% leaflet, whereas the *afaf tactac* leaf is composed of only 27% *tac* leaflet. Approximately four nodes above the first pod-bearing node, the percentage of leaf composed of either *tac* leaflet or normal leaflet becomes quite similar.

Experiment 2

Foliage expression in Experiment 2 (Figure 2) followed a pattern similar to that mentioned for Experiment 1. The percentage of the total leaf weight due to leaflets in *AfAf TacTac* plants (averaged over genetic backgrounds) was explained by a linear model, although the R^2 value was low (0.31). The percentages of total leaf weight due to *tac* leaflets in the *AfAf tactac* foliage type and *tac* leaflets in the *afaf tactac* foliage type (averaged over genetic backgrounds) were explained by quadratic models. The quadratic response of *tac* leaflets and linear response of normal leaflets was also observed in Experiment 1. The leaf weight due to *tac* leaflets in *afaf tactac* plants was approximately 30% at node 10, but it increased to over 60% by node 22. The percentage of leaf weight due to *tac* leaflets in *AfAf tactac* plants was approximately 10% throughout ontogeny.

These results demonstrate two important features of the apparent change in *afaf tactac* plants. First, the developmental change is evident regardless of the degree of interplant competition. This change was observed both under high levels of interplant competition in the drill-plots of Experiment 1 and under minimal levels of interplant competition on widely-spaced wire trellises in Experiment 2. The effect of a minimally competitive environment, such as is found with wire trellises, on agronomic performance of mutant foliage types has been discussed (Goldman and Gritton 1991). Although those results demonstrated that *tac* was responsible for a larger total foliage area (which is generally correlated with leaf weight) in *afaf tactac* plants in a minimally competitive environment, there appears to be no effect of the degree of competitiveness upon the developmental change associated with *tac*. Second, no change in *tac* leaflet expression was noted in the *AfAf tactac* background. This finding suggests that the developmental change associated with *tac* occurs only in the presence of *af*. Thus it seems quite possible that the true change occurs in the "cancellation," as Marx (1989) has stated, of the typical effects of *af*.

Experiment 3

Due to the lack of senescence in greenhouse-grown plants, leaves beginning with the third node were present at leaf harvest. The third node in the *afaf tactac* foliage type is characterized by a small strap-shaped appendage (Marx 1987). Since it was difficult to classify this appendage, data collection began with the fourth node. The primary difference between the field and greenhouse studies was the lack of senescence in greenhouse-grown plants, which allowed for examination of leaves at those nodes where leaves are not normally present in field-grown plants. The percentage of leaf due to *tac* leaflets in *afaf tactac* plants was over 60% in the nodes just above node 4 and then rapidly dropped to approximately 30% by node 10 (Figure 3). This may reflect the tendency of *afaf tactac* plants to produce strap-shaped unifoliate appendages in nodes 3, 4, and 5. Thus, although a "U"-shaped curve was apparent for *tac* leaflets in *afaf tactac* plants, trends similar to those noted in the previous experiments were noted above node 12.

In Experiment 3, a pattern of foliage expression similar to that observed in Experiments 1 and 2 was revealed (Figure 3). The percentage of leaf due to *tac* leaflets in *afaf tactac* plants (averaged over genetic backgrounds) showed a clear increase after node 14, while the percentage of leaf due to normal leaflets in *AfAf TacTac* plants (averaged over genetic backgrounds) and

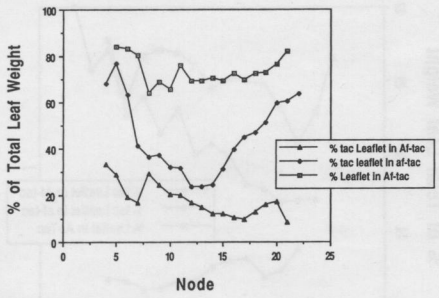


Figure 3. Percentage of total leaf weight due to *tac* leaflets in *afaf tactac* or *AfAf tactac* plants or normal leaflets in *AfAf TacTac* plants relative to node position. The response of *afaf tactac* is averaged over genetic backgrounds. The equation $y = 178.80 - 29.59$ Node $+ 1.35$ Node^2 describes the response of normal leaflets in *AfAf TacTac* plants. The equation $y = 119.15 - 13.58$ Node $+ 0.51$ Node^2 describes the response of *tac* leaflets in *afaf tactac* plants. The equation $y = 70.54 - 3.42$ Node $+ 0.11$ Node^2 describes the response of *tac* leaflets in *AfAf tactac* plants. The R^2 values for these equations were 0.88, 0.88, and 0.61, respectively.

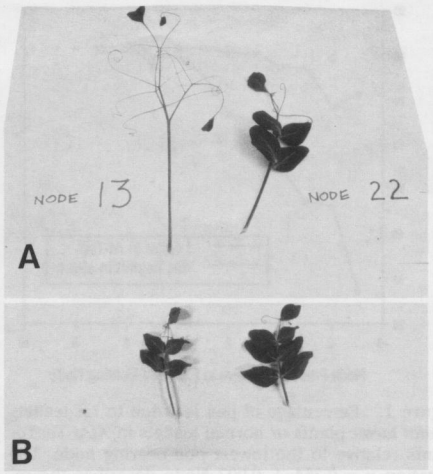


Figure 4. (**A**) Leaves from an *afaf tactac* plant at node 13 (*left*) and node 22 (*right*). (**B**) Leaves from an *afaf tactac* plant at node 22 (*left*) and an *AfAf tactac* plant (*right*). The *AfAf tactac* leaf shown is characteristic of all *AfAf tactac* leaves, regardless of node position.

tac leaflets in *AfAf tactac* plants remained fairly constant throughout ontogeny. As with Experiments I and 2, the percentage of leaf due to *tac* leaflet was approximately 30% at its lowest point, increasing to over 60% after node 20.

These experiments have demonstrated that a quantitative change in *tac* leaflet expression is present as development progresses in *afaf tactac* plants. A visual representation of this change is given in Figure 4A. The leaf in *afaf tactac* plants changes from a three-dimensional form at node 13 (left) to a more planar form at node 22 (right). Upper-region *afaf tactac* leaves look no different from the planar leaf form characteristic of *AfAf tactac* plants, shown on the right in Figure 4B. The leaf shown on the left in Figure 4B is an *afaf tactac* leaf from node 22.

The relationship between the first pod-bearing node and the change toward increased *tac* leaflet expression in *afaf tactac* plants, which was measured in Experiment 1, may be more than coincidental. The developmental change noted in this study seems to occur in the leaf at the node just slightly after the formation of the first pod. Since the shift from vegetative to reproductive phase is known to affect many developmental and physiological processes in plants, there may be reason to suspect that hormonal changes associated with reproduction have an effect on foliage gene expression. Although this characteristic was not measured in Experiments 2 and 3, data collected from identical genetic material grown in similar environments may be used for comparison since the lowest pod-bearing node remains relatively constant for a given variety. Averaged over genetic backgrounds, the lowest pod-bearing node for identical genetic material was 13.25 in environments identical to those used in Experiments 2 and 3 (Goldman and Gritton 1991, unpublished data). Data from Experiments 2 and 3 (Figures 2 and 3, respectively) reveal that the change noted in the percentage of total leaf weight due to *tac* leaflets in *afaf tactac* coincides approximately with the leaves formed above node 13. Further investigation of the possible causal role played by hormones in the expression of these genes should be conducted.

Conclusions

Results from the three experiments demonstrate that the *tac* allele in *afaf tactac* plants undergoes a quantitative change in expression as development progresses. The percentage of total leaf area due to *tac* leaflets in field-grown *afaf tactac* plants was 27% three nodes below the first pod-bearing node. By six nodes above the first pod-bearing node, the percentage of total leaf area due to *tac* leaflets was 84%. In contrast, the percentage of total leaf area due to leaflets in *AfAf TacTac* plants remained relatively constant at 75% throughout plant development (Experiment 1). Additionally, the percentage of total leaf weight due to *tac* leaflets in *AfAf tactac* plants changed little throughout plant development. In general, the percentage of total leaf area or total leaf weight due to leaflets in *AfAf TacTac* plants was explained by a linear model, while the percentage of total leaf area or weight due to *tac* leaflets in *afaf tactac* plants was explained by a quadratic model.

Up to the first several reproductive nodes, the *afaf tactac* leaf is characterized by a three-dimensional form. At higher nodes, the leaf takes on a planar two-dimensional form reminiscent of the *AfAf tactac* leaf. Stated in another way, the characteristic effect of the *af* allele is no longer present as development progresses. This change seems to coincide with the presence of the lowest pod-bearing node in drill-plot-grown plants. Trends in *tac* leaflet expression were similar in field-grown and greenhouse-grown plants. Agronomic performance and expression of the *afaf tactac* foliage type has been shown to differ depending on the degree of interplant competition (Goldman and Gritton 1991; Goldman et al. 1991). Results of this experiment, however, demonstrate that the percentage of the leaf due to *tac* leaflets does not appear to be affected by the degree of interplant competition.

Genes that alter morphology of the pea leaf provide the scientist with an excellent opportunity to investigate developmental processes in higher plants. In addition, the *af* and *tac* system of mutant genes may provide macromorphological clues to complex genetic events.

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