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ALMOND FLOWER DEVELOPMENT

Timing of Floral Differentiation

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Introduction

Flower development in almond, as is typical of most deciduous tree species, occurs during the growing season prior to bloom. The timing of these events varies widely among species, and even among cultivars within species. A comprehensive knowledge of timing of flower development is fundamental to informed orchard management decision-making. The information becomes especially important in managing stress. Current growth and cropping conditions are readily apparent and it is often clear how timely management of stress conditions can influence the tree and crop at this level. However, the concealed nature of events occurring in buds that lead to floral development makes it more difficult to appreciate the timing of critical stages and the potential impact of stresses on those critical developmental processes. This is illustrated by the work of Goldhamer and his colleagues (summarized in Almond Board of California, *Years of Discovery*, 1999) who found that stresses incurred following deficit irrigation regimes affected subsequent years' crops. Their findings implicate stresses during critical stages of flower bud development and differentiation.

When we began this project three years ago, the current understanding of flower bud differentiation was inadequate. Almond flower differentiation has not been investigated since the classical investigations of Tufts and Morrow (1925) and Brooks (1940), both of whom investigated differentiation in 'Nonpareil' in the Davis area. These results used now-obsolete methodologies and were focused in a growing area that inadequately represents the range of almond production in California today. We initiated a study using scanning electron microscopy to update these early findings and to extend them to the major almond-growing areas in California. After three years' data accumulation, we have begun to put together a picture of the phenology of almond flower bud differentiation. What we have found is that the results of earlier workers is either inaccurate or nonspecific enough to be misleading to those who would predicate research or orchard-management decisions on them.

Methods

We collected bud samples of 'Nonpareil', 'Carmel', and 'Butte' from three major almond growing areas — Northern Sacramento Valley (Chico, Butte County), Northern San Joaquin Valley (Modesto, Stanislaus County), and Southern San Joaquin Valley (Shafter, Kern County) — and from the UC Davis orchards at Davis ('Nonpareil) and Winters ('Butte', 'Carmel') in 1997 and 1998, and samples of 'Nonpareil' from Davis in 1999. Well-managed commercial or experimental orchards were selected.

In order to generate the most useful data, we focused primarily on 'Nonpareil' with secondary emphasis on the other two cultivars. Samples from Butte, Stanislaus and Kern Counties were collected and shipped to Davis via overnight express delivery. In 1997, collections were made weekly. 'Nonpareil' was collected from each site on each collection date. We collected 'Carmel' on a regular basis from Butte and Stanislaus counties and 'Butte' on a regular basis from Kern county. We collected 'Butte' from the northern locations and 'Carmel' from the south so that we could have material for comparisons, but we did so less frequently. In 1998, we made our collections every other week and included all three cultivars on each collection date. In 1999 collections were made weekly. In all cases, collections were stopped when more than half of the samples for a given week had attained the stage of development where the pistil primordia were initiated.

Potentially reproductive buds were identified on the basis of position on the shoot. Twenty buds each from the north and south sides of the trees were dissected to reveal the developing shoot apex within the bud. The dissected buds were prepared for scanning electron microscopy using standard practices. Briefly, material was fixed in 3% glutaraldehyde in phosphate buffer, pH 6.8, at 4°C for 3 to 4 weeks, dehydrated in a graded ethanol series to dry amyl acetate, critical point dried using CO_2 as a transitional fluid, sputter-coated with 30-40nm gold and observed in the scanning electron microscope. The scanning electron microscope images were captured as digital image files, stored on a computer and scored for developmental stage.

Results

Floral development is a sequential series of events. The growing point (meristem) of the bud begins its activity creating vegetative organs, the bud scales. At some point it undergoes a transition to reproductive development. This transition is marked by the production of three bracts (small, leaf-like organs that subtend the flowers) and a subsequent change in the three-dimensional geometry of the meristem leading to flower initiation. The floral meristems then produce organs in sequence: sepals, petals, stamens and pistils. We classified the reproductive buds according to eight stages of development at the reproductive apex as described in Table 1. Note that we have modified the numbering and descriptions of the stages from that reported in previous years. Our current terminology more accurately reflects the stages of bud differentiation as we understand them at this time.

It is our view that orchard managers should take special note of stages 1 and 2, leading to floral initiation, and stages 6 and 7, leading to pistil initiation, as key stages in the development of the following year's crop. We also feel that stages 1 and 6 are most informative in regard to orchard management practices. Stage 1 marks the transition from a vegetative to a reproductive state at the bud apex, and stage 6 indicates the onset of readiness to initiate a pistil at the floral apex.

Table 1. Developmental stages of almond buds. Stage numbers in this table are referred to in the results. Note that we have revised the terminology and identification of the stages relative to previous reports.

Number	Developmental Stage	Developmental Activity
0	Vegetative (Pre-reproductive)	Bud scales are produced at the apex.
1	Transition to Reproductive Stage	Increase in meristem size.
2	Flower Initiation	Apex forms elongate, broad dome. Bracts form.
3	Sepal Initiation	Sequential initiation of five sepal primordia.
4	Petal Initiation	Sequential initiation of five petal primordia.
5	Stamen Initiation	Sequential initiation of multiple stamen primordia.
6	Transitional/Pre-Pistil Initiation	Stamen initiation complete, concavity apparent at apex.
7	Pistil Initiation	Pistil primordium visible at the center of the apex.

Our results indicate that development is occurring substantially earlier than had been suggested in the older literature. This may be a consequence of our experimental methods. Improvements in digital imaging and data collection techniques have enabled us to observe large numbers of buds, many more than had been possible even a short time ago. Additionally, subtle developmental events that likely escaped the notice of previous workers are detectable using the higher resolution methodologies we employed here. We have carefully reviewed the existing literature. It is clear that earlier workers had not recognized the earliest stages of flower initiation. Tufts and Morrow's (1925) work considers stages that correspond to sepal initiation to reflect floral initiation.

The impact of year to year differences is evident from our analyses. Although evaluations are not complete, it is clear that flower development, similar to all other tree-development events, has been delayed in 1998 relative to 1997. 1998 was an unusual weather year in several regards. The

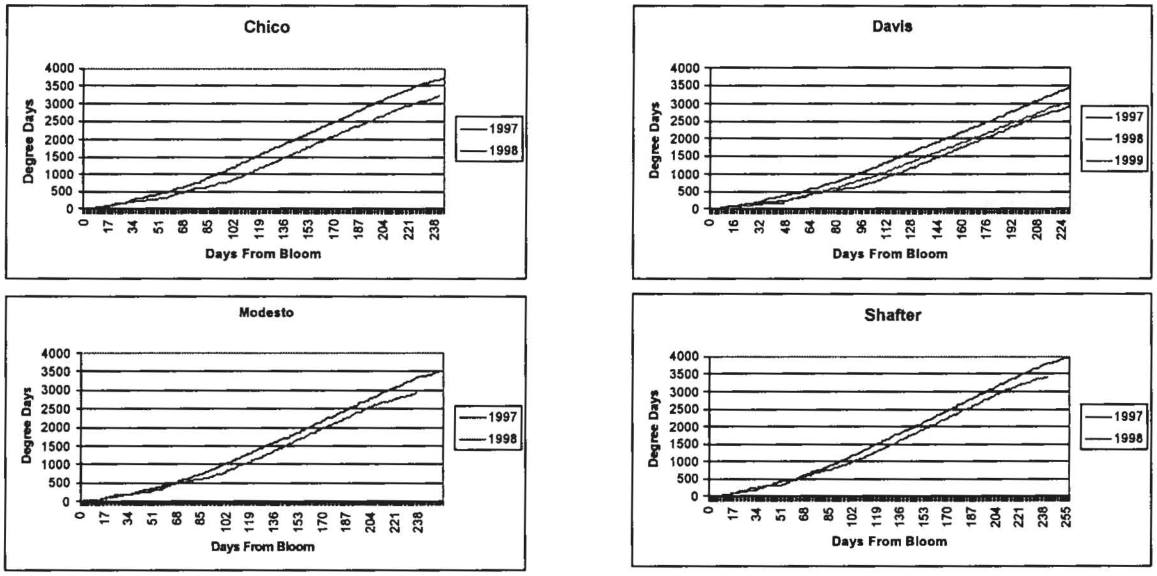
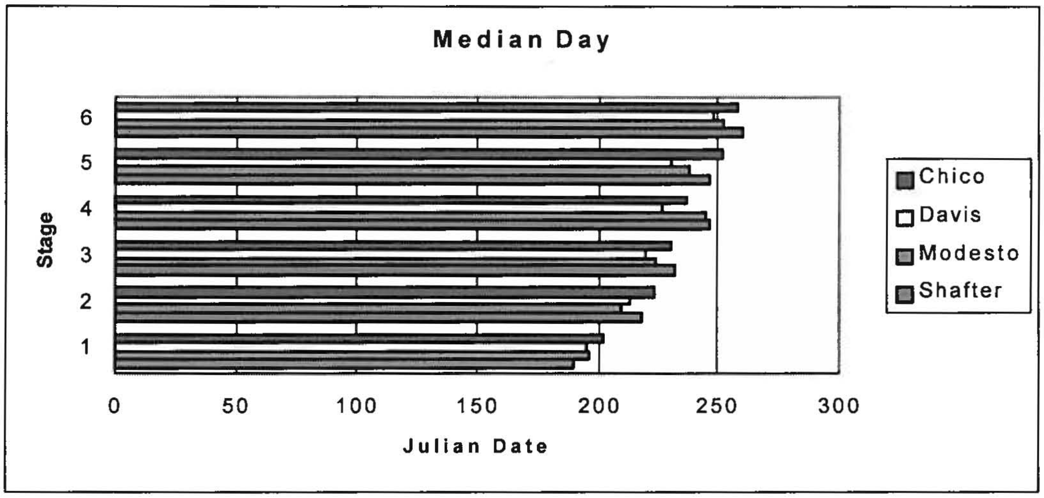


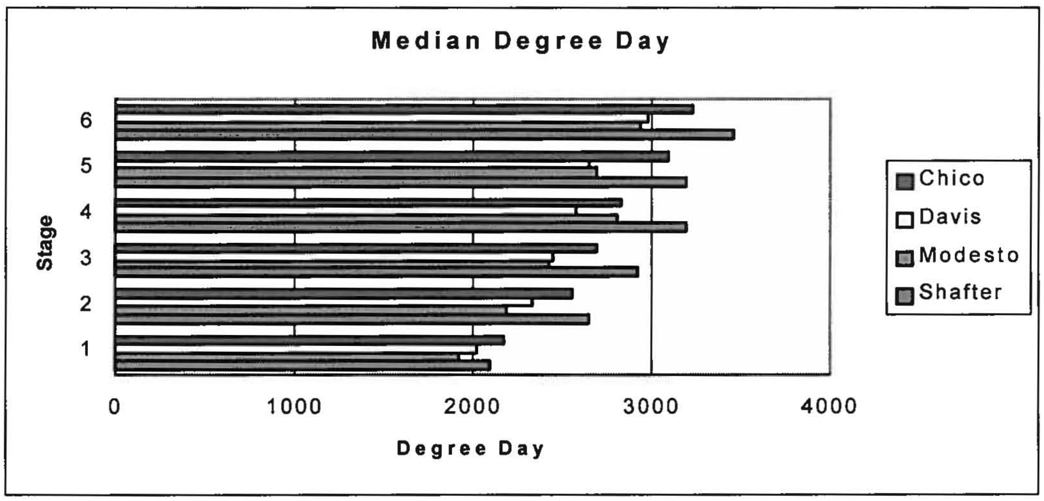
Figure 1-4. Accumulated degree-days vs. time after 'Nonpareil' bloom in each of the collection locations for 1997-99.

El Nino weather pattern produced a cool spring and early summer followed by a hot mid to late summer. This may be seen in the charts where degree-days are plotted against calendar days during the growing season.

As a result, flowering and harvest for 1998 and 1999 were exceptionally late in almond, as in nearly all tree crop species. We found similar effects in flower development, where the occurrences of the various stages are running 2 to 4 weeks later than 1997. It is interesting to note that these results are somewhat more comparable to what had been reported in the earlier literature when we correct the inferences previous workers made about stages of development to correspond to what our results have shown.. We have not investigated the weather patterns that characterized the years in which Tufts and Morrow's or Brooks's studies were made.

The following figures illustrate median time to each of the bud developmental stages for each collection.





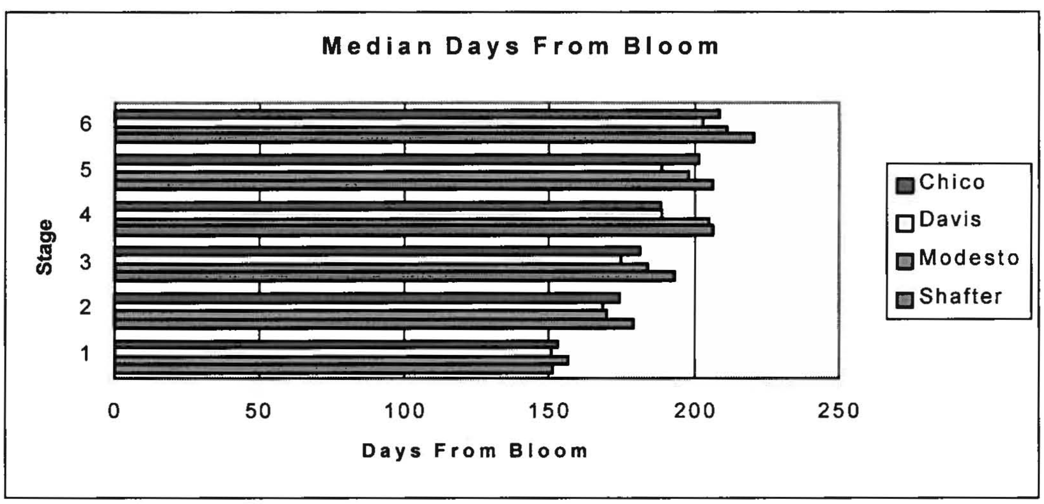
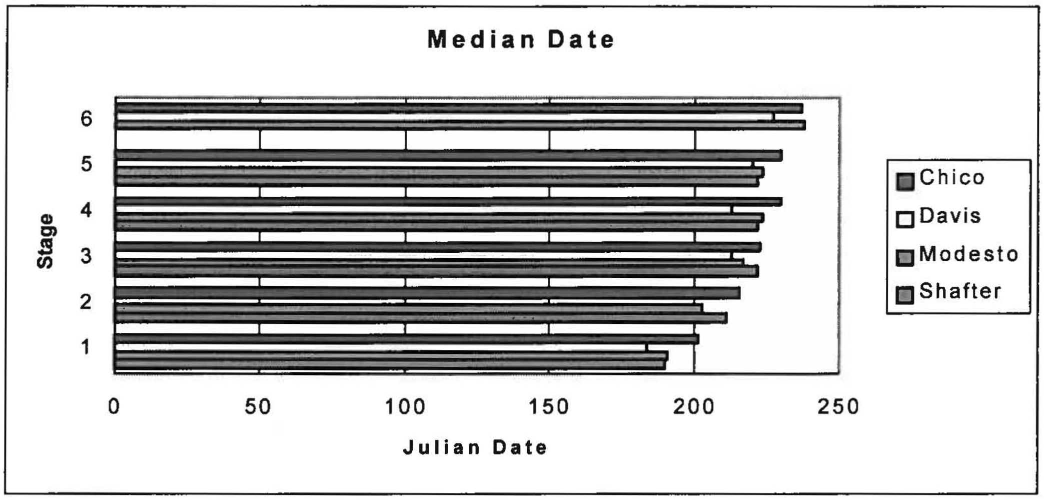
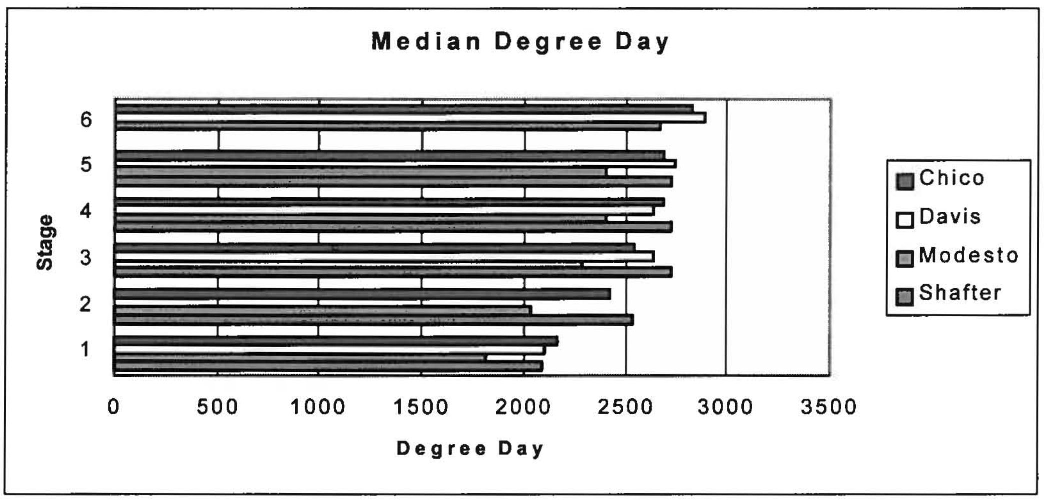


Figure 5. 'Nonpareil' 1997 Data.





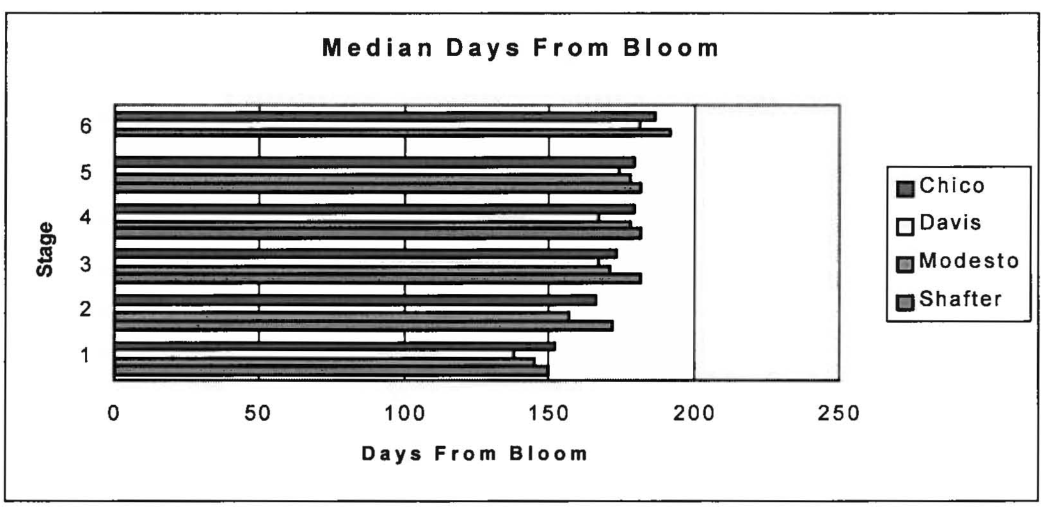


Figure 6. 'Carmel' 1997 Data.

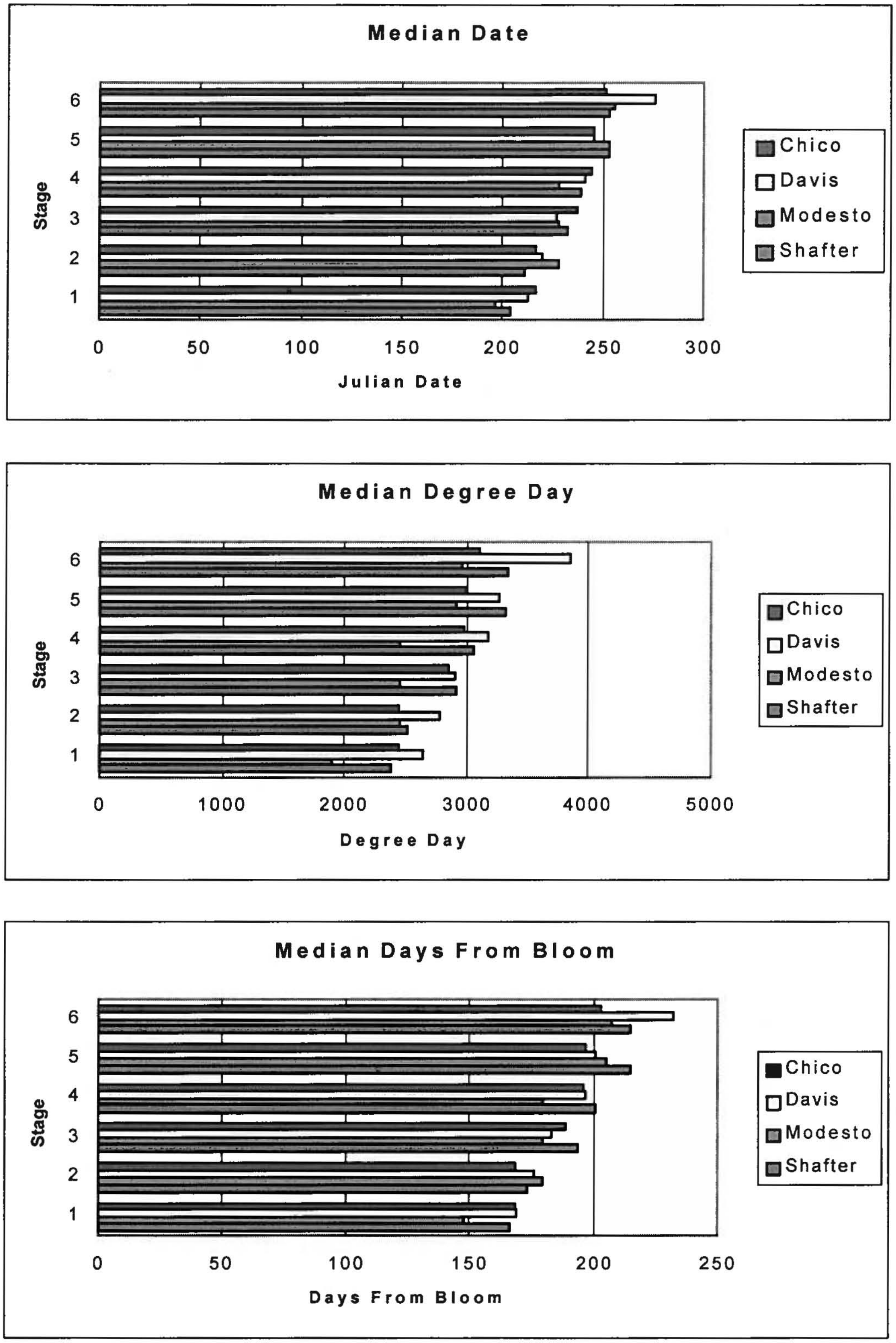
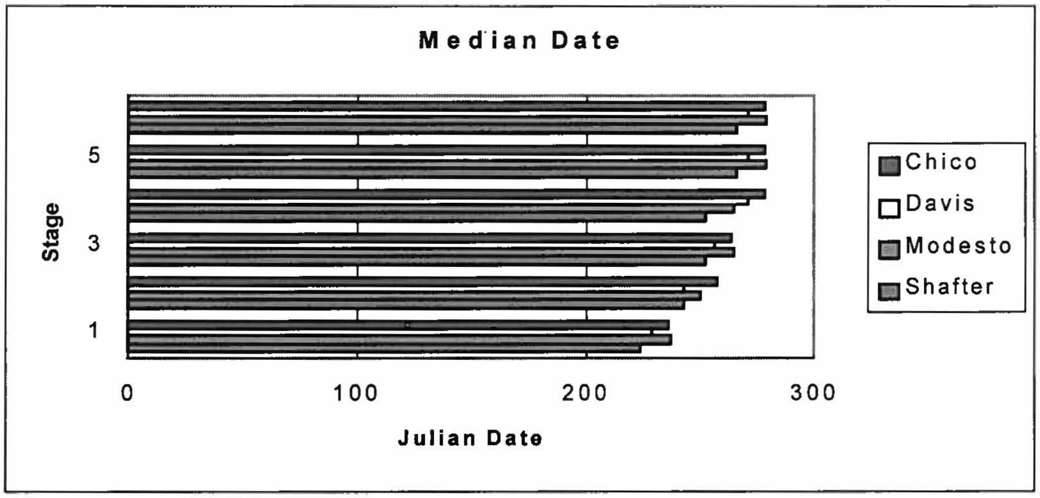
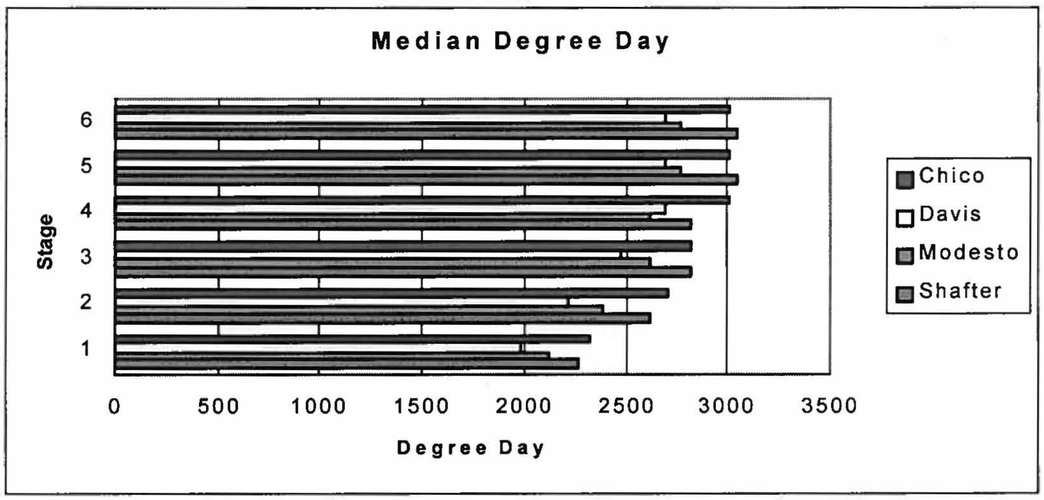


Figure 7. 'Butte' 1997 Data.





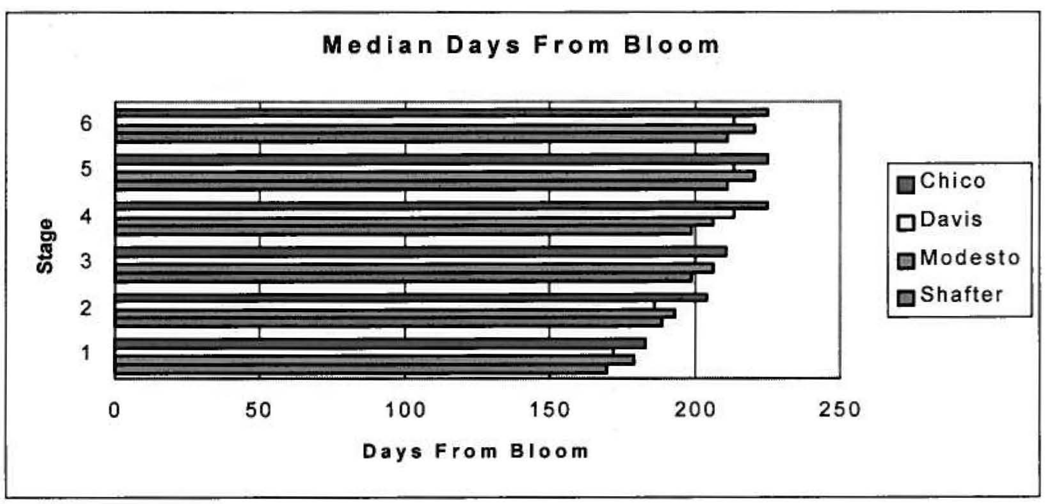
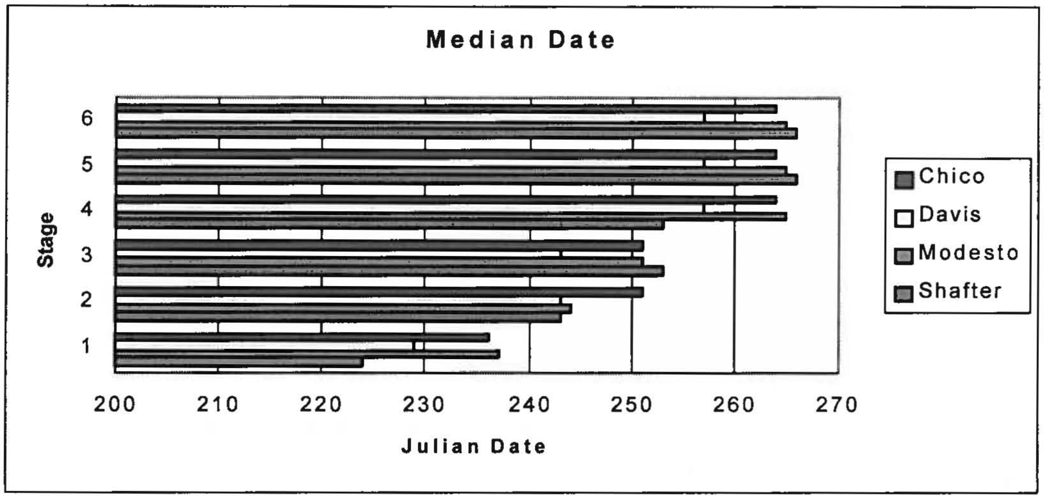
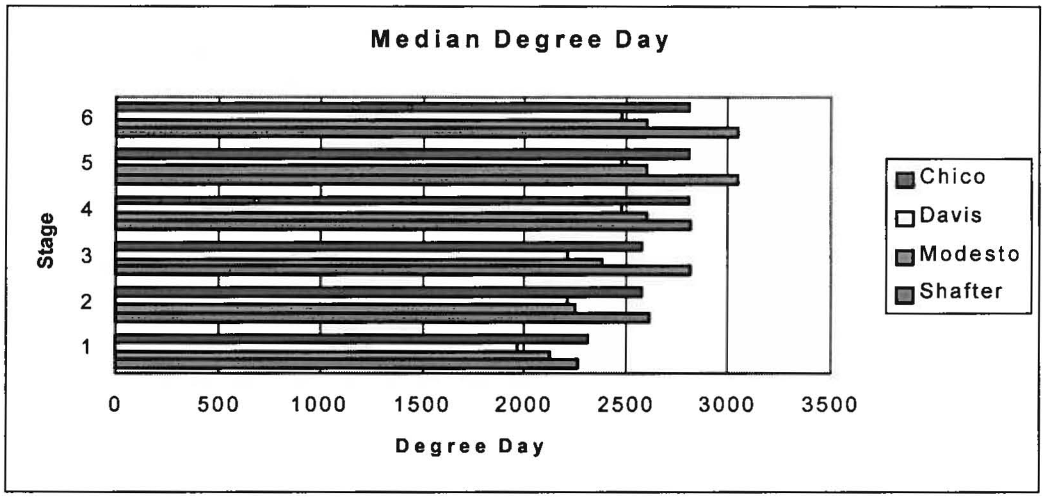


Figure 8. 'Nonpareil' 1998 Data.





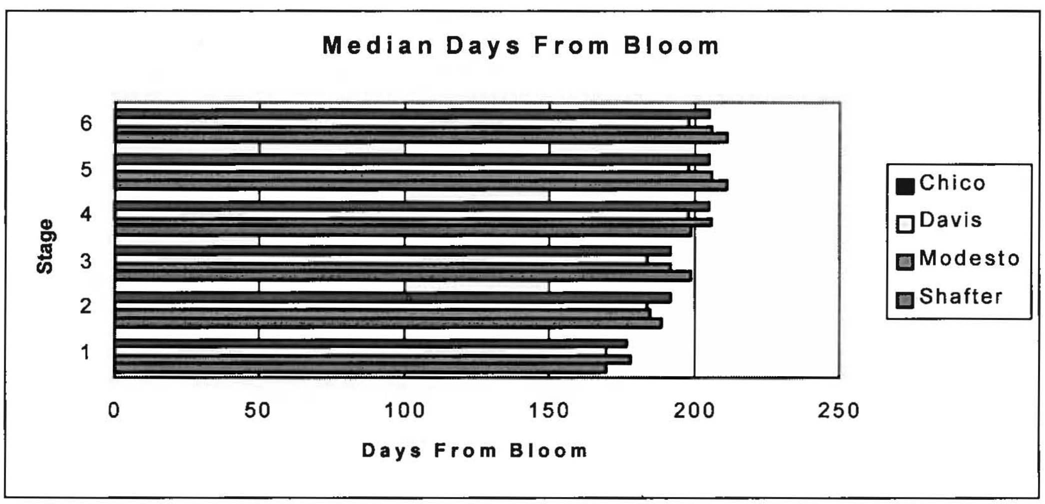
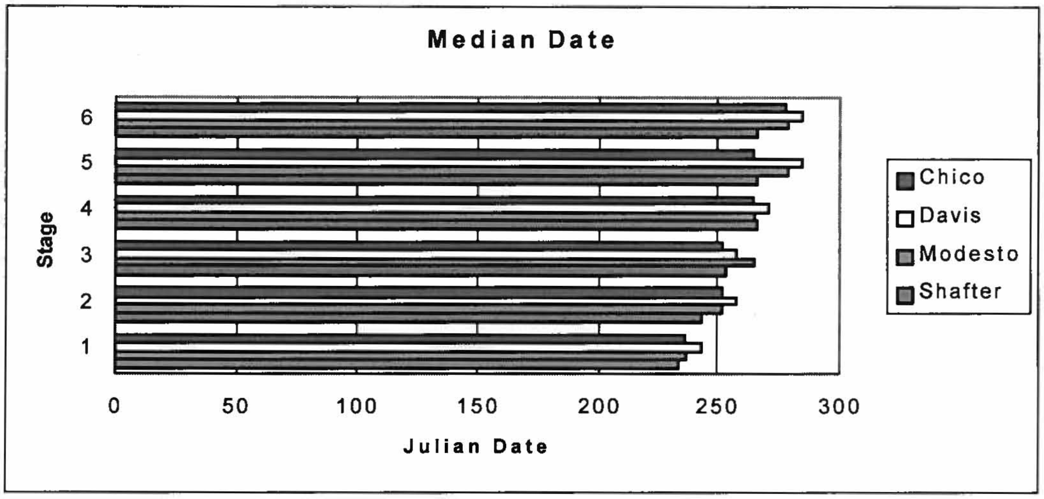
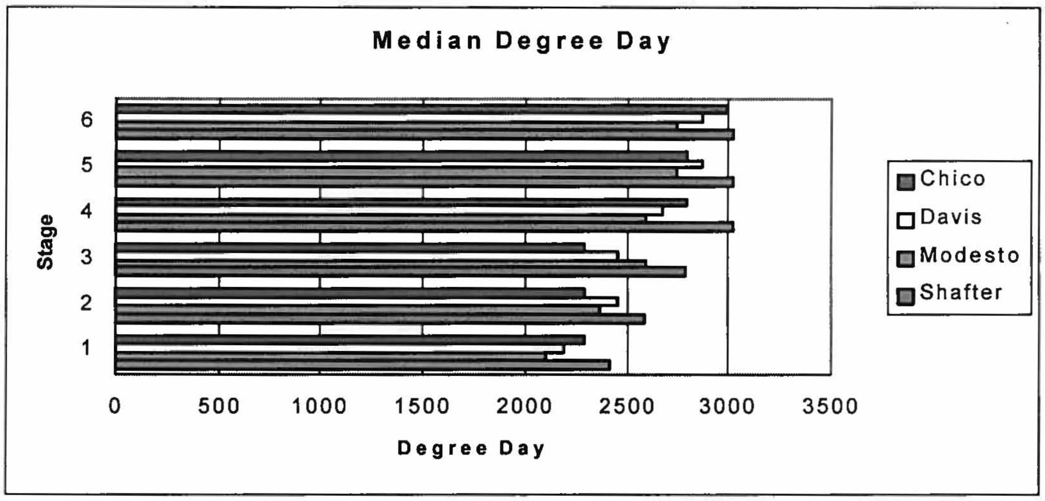


Figure 9. ‘Carmel’ 1998 Data.





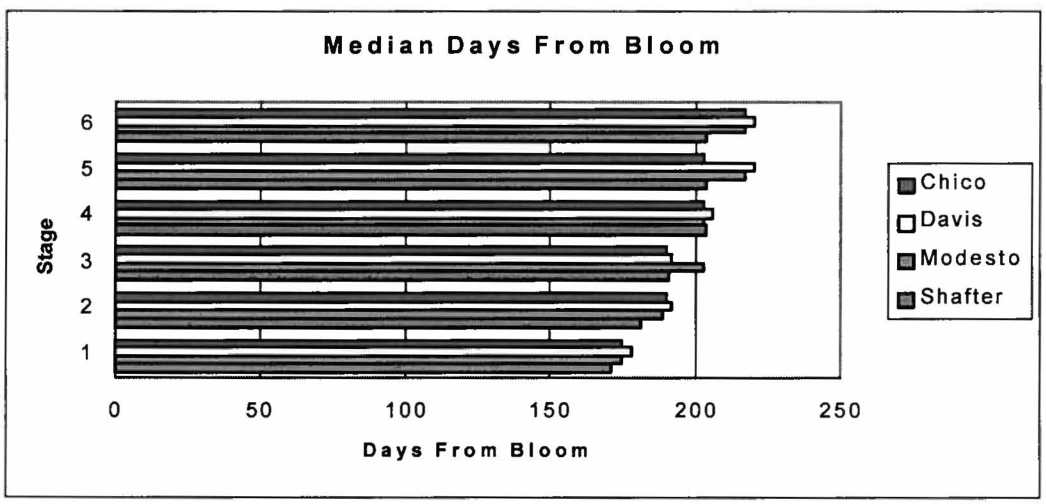


Figure 10. 'Butte' 1998 Data.

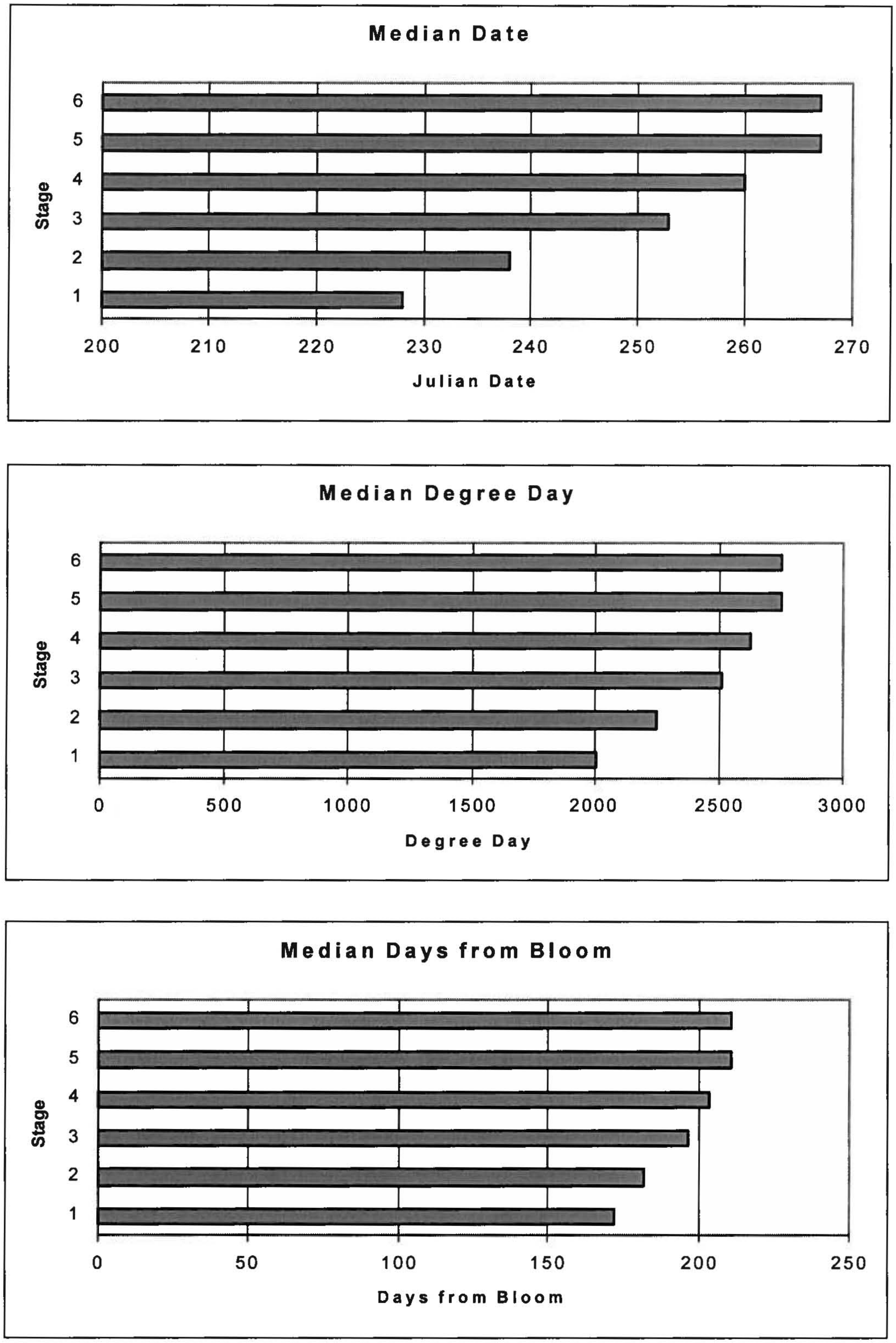


Figure 11. 'Nonpareil' in Davis, 1999.

We are currently fitting the data we have to logistic and probit modes that we anticipate will allow predictions of stages of bud differentiation based on degree-days and time from bloom. Our work is focused on stages 1 and 6 which best describe readiness to initiate flowers and to initiate pistils, respectively.

These analyses have revealed several important points: There is a significant effect of aspect such that the north sides of trees are delayed relative to the south sides. There are, as expected, significant differences among varieties. The rate of maturation is more rapid in some locations. When degree-day and aspect interaction and degree-day and year interactions are excluded, both the probit and the logistic models converge for stages 1 and 6. Results indicate that accumulated degree-days affect these two stages of flower development.