Assessing the Carbon Budget of Almond Trees and Developing a 3-D Computer Simulation Model of Almond Tree Architectural Growth and Dry Matter Partitioning

Project No.:   **10-PREC1-DeJong**

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Objectives:

This project has two major objectives. The first is to review the available research data that has been collected in California on almond (and related species) tree growth; biomass productivity; dry matter partitioning; and carbon and nitrogen assimilation, utilization and distribution to develop quantitative estimates of standing tree biomass in California almond orchards.

The second and longer term objective is to develop a comprehensive, functional-structural tree model of almond tree architectural development, growth, and carbon partitioning/source-sink interactions within the tree. This model will simulate growth and physiological responses to light distribution within the canopy and daily temperature changes as well as responses to user imposed pruning practices.

Eventually, pertinent data from both of these objectives will be compiled and provided to Johan Six's laboratory for greenhouse gas modeling purposes.

Interpretive Summary:

Objective one:
A review of available literature on tree growth; dry matter partitioning and biomass productivity of almond trees over multiple years indicated that there is not enough published data to make reliable estimates of the amount of carbon contained in the standing biomass of mature almond orchards at this time. However in the past several years it has become standard practice to engage professional tree removal companies to remove almond orchards at the end of their productive life. Since these companies haul and weigh the chippings subsequent to the removal of an orchard, reasonable

estimates of orchard standing biomass should be available from these operations. We have contacted several of these companies and requested their cooperation in sharing data resulting from orchard removal. However we have had difficulties in obtaining detailed data because of information privacy issues but we are working to solve those issues by directly contacting growers who have recently removed orchards.

Objective two:
Almond tree growth and yield is dependent on a complex set of interactions involving the plant genotype, the physiological and developmental processes that occur within the tree, the interaction of these processes with the environment that the tree grows in, and responses to horticultural manipulation of the tree by the grower. Understanding the carbon budget, growth and yield responses of perennial crops like almond are even more complex than most crops because the effects of all these factors are carried out over multiple years.

Recent advances in computer technology have made it possible to develop functional-structural plant models that simultaneously simulate whole tree photosynthesis, tree architectural growth and carbon partitioning within the structure of the tree, and display tree structural development in three dimensions on a computer screen. The most advanced of these types of models is the L-Peach model that has been developed to simulate peach tree growth and development. A long-term objective of this project is to continue the development of the L-Peach model and convert it to an L-Almond model.

The first step for conversion of the L-Peach model to an L-Almond model was to develop statistical models to describe patterns of buds that occur along Nonpareil almond shoots of different lengths. Development of these statistical shoot bud fate models for Nonpareil are now complete. The field data collection phase of additional field studies to develop detailed statistical shoot models to describe shoots of cultivars with contrasting growth habits (Nonpareil, Aldrich and Winters) and to study shoot architectural responses to water stress and pruning has also been completed. Data analysis and construction of additional bud fate models from these studies will be completed in the fall of 2011.

The second step in the modeling project was to begin converting the L-Peach model into an L-Almond model by inserting leaf photosynthetic characteristics of almond trees and the statistical models of almond shoots into the L-Peach model. Preliminary work on this was done in 2008 and this exercise documented that it could be done. Since more robust statistical shoot models were completed in June, 2011, work on using these new shoot models to develop the L-Almond model has now begun.

While waiting on the almond shoot bud fate models we have completed research to incorporate water transport within the tree structures generated by the L-Peach model so that hourly values for water potential can be calculated each hour for every node within the structure of the simulated trees. The hourly water potential values calculated by the model now interact with the physiological functioning of the trees so that tree growth and yield responses to irrigation scheduling can also be simulated by the model. Development of an integrated dynamic simulation model of almond tree growth and

productivity is a challenging project but will result in the most sophisticated environmental physiology-based model of a fruit or nut tree ever developed.

A corollary effort associated with this project has been the analysis of data from the spur dynamics study carried out by Dr. Lampinen's laboratory from 2001 to 2007, in order to develop data on long-term spur behaviour that can be used in the L-Almond model. This research has provided valuable insights into the fruit production behaviour of almond tree spurs that are useful for general understanding of almond tree productive behaviour as well as for developing the L-Almond model. A scientific paper describing the relationships between previous year leaf area and spur behaviour has been accepted for publication (Lampinen et al. 2011).

Materials and Methods:

Assembling Data on Whole Tree Biomass

Several biomass companies have been contacted to obtain standing biomass data at the time of orchard removal. We began to compile data on locations of removed orchards, age of orchards at the time of removal, spacing of the trees and cultivars involved. However, we have had limited success because of concerns that the orchard removal companies have over issues of confidentiality of grower information. Thus we have recently altered our approach and are now attempting to collect these type of data by contacting growers directly and asking them to complete survey forms that can then be used to get additional data from orchard removal companies if needed. We have begun the process of surveying large grower organizations but it is too early to report results. We believe that this approach will provide good estimates of standing biomass of mature almond orchards and will be useful for estimating the amount of carbon stored in trees in California almond orchards.

Development of an L-Almond Model

The second objective (developing a model of almond tree growth) began with statistically analyzing the structural patterns of various sizes of almond shoots using Hidden Semi-Markov Chain (HSMC) analysis techniques (Guedon et al. 2001). The almond shoot study was conducted in a 3-year-old orchard located in Sutter, California. In this orchard, three almond cultivars with different tree architectures ('Nonpareil', 'Aldrich', and 'Winters') were evaluated however shoot models have only been completed for the Nonpareil cultivar. After observing 1-year-old proleptic shoots (shoots that grow from over-wintering buds) in the tree canopy, five categories of shoots (based on the length of shoots) were pre-established. The 1-year-old proleptic shoot categories were: long shoots (more than 1 meter); medium–long shoots (between 60 cm and 100 cm); medium shoots (between 20 cm and 60 cm); medium–short shoots (between 5 cm and 20 cm); short shoots (less than 5 cm). Before bloom in 2009, 40 shoots of each shoot category were tagged in 10 trees. That is, in every quarter of a tree, 1 shoot of every category was selected. In addition, 40 epicormic shoots (water sprouts) were also tagged. They were mainly located in the center of the tree canopies.

The structure of every shoot was evaluated by recording two variables at every node: the fate of the lateral meristems and the number of lateral flower buds. Data were collected from the base to the tip of the shoot, in the same way as the shoot developed. For the meristem fate evaluation, each node was placed into one of the following categories: blind, central floral bud, central vegetative bud, or sylleptic shoot (lateral shoot growing from a main growing shoot). For the number of flower buds per node, the axillary flower buds were registered as well as the number of flower buds growing on the sylleptic shoots.

The two variables were coded and arranged as a bivariate discrete sequence of data that represent the observations at each node along the shoots. The bud fate variable was coded using increasing numbers from 0 to 3 to indicate the increment of vigor from one observation to another (0: blind; 1: floral bud; 2: vegetative bud; 3: sylleptic shoot). The location of the terminal bud was also registered and was coded as 4. The second variable that represents the number of flower buds per node was coded from 0 to 3. 0, 1, or 2 flower buds were the more frequent numbers per node. Three or more flower buds per node were less frequent; therefore when 3 or more flowers were observed in a node, they were coded as 3.

The sequences were analyzed using V-Plant.AML software, originally called AMAPmod (Godin *et al.*, 1997). The data were as previously described by Costes and Guédon (1997) and Guédon *et al.* (2001). Intensity distributions represent the empirical distribution of the different observations at each node rank (**Figure 1**). From the intensity distributions of bud fate and number of flower buds, it was possible to identify zones along the shoots with different frequencies of observations between zones. These types of sequences have been modeled in other species by hidden semi-Markov models (HSMC) (Costes and Guédon, 1996; Fournier *et al.*, 1998; Costes and Guédon, 2002). In this type of model, the number and succession of states are represented by a Markov chain. If the length of the states can be described by occupancy distribution, then the model is a semi-Markov chain. Because the observations within a state are not homogenous, observation distributions are attached to each state of the semi-Markov chain to indicate the probability of each observation within a state. Then, the complete model is a hidden semi-Markov chain (Costes and Guédon, 2002). In this study, the successions of states were defined by the initial probabilities that determined the first zone of the shoots and by the transition probabilities that described the succession of zones along the shoot. The occupancy distributions represent the length of each zone of the shoots expressed as the number of nodes. Two observation distributions in each zone describe the fate of the central bud and the number of flower buds per node.

Based on the analysis of the empirical distributions, initial models were built with hypotheses of the number of zones in the shoots, the direction of the sequences to be described, and the presence of a given observation in each zone. The V-Plant.AML software estimated the parameters of the theoretical models using an iterative algorithm which maximized the likelihood of the observed sequences starting with the hypotheses established in the initial models. Theoretical distributions were plotted along with the empirical distributions to evaluate estimated models (Guédon *et al.*, 2001) (**Figure 1**).

The data collection phases of additional field studies were completed in spring of 2011 to evaluate the influence of water stress, cultivar (genotype), and severity of pruning on the structure of different size categories of almond shoots using HSMC analysis. However the data analysis and shoot bud fate models from these studies won't be completed until next year. These studies will be used as the basis for developing more robust sub-models of almond trees growing under different pruning practices and irrigation management strategies. Collection of data on the influence of water stress on shoot growth characteristics of Nonpareil trees was initiated in an ongoing field experiment of Dr. Lampinen (USDA-Pacific Area-Wide Pest Management Program for Methyl Bromide Alternatives-Almonds & Stone Fruits). Data on shoot growth (rates of node initiation and shoot length growth), shoot structural characteristics, and tree water potential were collected bi-weekly during the growing season. An additional field study was conducted in a young orchard near Davis to study the effects of severity of winter pruning on the structure of shoots that grow in the subsequent year.

We have also spent substantial effort improving the foundational software of the L-Peach/L-Almond simulation model (Allen et al. 2005; Lopez et al. 2008). The original models used daily steps to simulate physiology and growth but this significantly limited the ability to simulate detailed physiological responses to environmental factors such as temperature and plant water potential. Therefore we changed the model to function on hourly time steps, and more recently have successfully improved the model so that a circuit controlling the uptake and transport of water by the plant can be simulated simultaneously with carbon uptake and transport. The details of how this was done have been described in DaSilva et al (in press).

A subproject was undertaken to develop a more complete understanding of factors that influence the behavior of individual almond spurs within canopies of Nonpareil almond trees. For this study we analyzed "spur dynamics" data previously collected by Dr. Lampinen's laboratory. In the spur dynamics study individual spur behavior was monitored on 48 trees in a 150 acre orchard that was planted in 1996. Tree spacing was 24 ft. between and 20 ft. within rows. Cultivar composition was 50% 'Nonpareil' with 25% 'Monterey' and 25% 'Wood Colony' as pollenizers (rows of Nonpareil trees planted with alternating rows of the pollenizer cultivars). The orchard was divided into four equal-sized replicate blocks and fifty spurs were tagged on twelve 'Nonpareil' trees within each of the four blocks. A total of 2,400 spurs were tagged with aluminum tags in late March and early April 2001. Twelve spurs were selected on each of the North-East and North-West quadrants of individual trees, and 13 spurs were selected on each of the South-East and South-West quadrants of the same trees. Tagged spurs were located at positions ranging from shaded (near the trunk) to exposed (on the periphery) portions of the canopy at a height of ~ 6-10 ft.

The dynamics of annual growth, flowering, fruitfulness, and spur mortality were quantified using annual assessments of tagged spurs. In July 2001 the numbers of fruit and leaves per tagged spur were counted and relative leaf sizes were noted. In addition, a similar spur from a nearby location (but not so near as to be a direct influence on the tagged spur) with a similar light exposure was sampled for leaf area and leaf specific weight analysis. Leaf area was assessed by taking digital photographs of all of the

leaves on each spur. Leaves were then dried and weighed. Leaf specific area (leaf area per unit dry weight) was calculated from leaf area and leaf dry weight measurements. In July 2002, the numbers of flowers, fruit and leaves per tagged spur were counted and leaf sizes rated. Adjacent spurs were sampled for leaf area/dry weight analysis as in 2001. In July 2003, 2004, 2005 and 2006 the numbers of flowers, fruit and leaves per tagged spur were again counted. Leaf size was estimated from leaf length measurements by developing regression equations relating the length of the longest leaf on each spur with the leaf area of spurs (using an independent sample of 400 spurs from the range of locations within the canopy). Adjacent spurs were sampled again in July 2004, for leaf area/dry weight analysis from all 48 monitored trees. In July 2005, adjacent spurs were sampled from two trees per replication for a total of eight trees. In July 2006 and 2007, adjacent spurs were again sampled from all 48 monitored trees. Specific leaf area was calculated as the spur leaf area divided the dry weight of leaves. The number of flowers produced on each tagged spur was counted in the spring of each year from 2002 through 2007.

Data from this long term "spur dynamics" trial were used to assess the association between spur leaf characteristics, spur flowering, and fruit production characteristics, and to analyze the tendency of almond spurs and trees to alternate bear over multiple years.

Results and Discussion:

Assembling Data on Whole Tree Biomass
As stated above this aspect of the project has not been successful up to the present because we have not received data on enough removed orchards to make robust estimations of almond orchard standing biomass. We have adjusted our approach to collecting the data and plan to obtain adequate data to make robust estimations during 2011-12.

Development of an L-Almond Model
To begin meeting the second objective of this project we began work to convert the existing L-Peach model into an L-Almond model. Because perennial crop growth is so complex and dynamic over long periods of time, there have been very few quantitative studies of tree growth dynamics and carbon budgets over time. The work that has been done in California has come mainly from the DeJong laboratory (Grossman and DeJong 1994, Esparza et al. 1999, Esparza et al. 2001, Rufat and DeJong 2001). However this research needed to be expanded to provide a more dynamic and accurate picture of tree growth and resource utilization dynamics at specific periods during the growing season.

The first step for converting the L-Peach model to an L-Almond model was to develop statistical hidden semi-Markov chain (HSMC) models to describe patterns of buds that occur along Nonpareil almond shoots of different lengths. An adequate set of HSMC shoot models for Nonpareil have been developed based on completion of field studies in the spring of 2011 (**Figure 1**). **Figure 1** is a diagrammatic example of the structure of one category of Nonpareil shoot. It shows that the shoot can be statistically divided into rather well-defined zones based on the bud fates at each of the nodes along the shoot.

Similar models have been developed for each of five categories of proleptic shoots (shoots that grow from over-wintering buds) and for epicormic shoots (water sprouts). These HSMC models will now be used to define shoot structures in the larger L-Almond model that is under development. Construction of HSMC models is a very efficient approach for quantitatively describing shoot structural differences among cultivars and in response to imposed treatments, so additional HSMC shoot models are being constructed and analyzed to characterize shoots of cultivars with differing growth habits (Nonpareil, Winters and Aldridge), water stress conditions during the growing season, and dormant pruning severities. However it is too early to report on these efforts.

Attempts to improve the foundational software of the L-Peach/L-Almond simulation model (Allen et al. 2005; Lopez et al. 2008) have been very successful. This involved many theoretical calculations to successfully adapt the models to run on hourly time-steps and incorporate water transport within the modeled tree structures. The model can now calculate daily courses of water potential at every node within the structure of simulated trees. Last year we presented preliminary results of sample daily patterns of stem water potential and the effects of simulated water stress on leaf growth in the L-Peach model. Subsequently this research was able to provide examples of how differences in irrigation frequency (thus periods of short-term water stress) over multiple years can influence long term growth and productivity in peach trees (**Figure 2**). Although this simulation was run with the L-Peach model it demonstrates the type of simulations that will be possible when L-Peach is converted to an L-Almond model. This is the first model of its kind that has successfully modeled both carbon and water transport in a "virtual plant" and it opens up the possibility to explore simultaneous interactions between photosynthesis, individual organ growth, dry matter partitioning and plant water stress in ways that were never previously possible.

The development of an integrated dynamic simulation model of almond tree growth and productivity is well on its way and when completed will result in the most sophisticated environmental physiology-based model of a nut tree ever developed. We believe that it will provide new, unique insights into factors affecting the growth and yield of almond trees as well as provide the basis for estimating the carbon sequestered in the standing biomass of almond orchards.

Modeling Spur Dynamics
Last year we presented preliminary data on the relationships between previous year leaf area and current season flowering and fruiting. The analysis of these aspects of "spur dynamics" is now complete and a paper detailing the results has recently been accepted for publication (see Lampinen et al, 2011). Briefly this study documented that previous year spur leaf area is strongly related to spur viability and flowering. The greater the leaf area in the previous year, the higher the probability of spur survival into the next year and the higher the probability for the spur to bear one or more flowers. Previous year bearing also appears to influence viability and return bloom, especially in spurs with low leaf area. These results suggest that spur source-sink balance is basic to the life cycle of almond spurs. Furthermore, the results are consistent with the hypothesis that spurs are semi-autonomous organs with respect to carbohydrate balance for much of the growing season. This study also showed that average annual

spur mortality in the early orchard years was about 4% but that increased to about 10% in later years. It also indicated that it is relatively rare for spurs to bear fruit in two consecutive years.

The fact that it is rare for a spur to produce fruit in two consecutive years induced us to do a follow-up study to analyze the tendency for alternate bearing in almond at multiple levels (spur, individual tree, and orchard). For this analysis we used data from the "spur dynamics" study and the UC "regional variety trial" (1997-2006) project also funded by the Almond Board of California. The results of these analyses have been written up and will be submitted to a scientific journal for publication. In summary this study showed that, while it is relatively rare that individual spurs bear fruit in two consecutive years, it does not necessarily lead to alternate bearing at the orchard level (**Figure 3**). The analysis indicated that this is because the percentage of spurs that bore fruit in a given year was relatively small relative to a large population of spurs sampled across multiple trees (<20%) (**Figure 4**). Thus there were always a large number of non-fruiting spurs available to bear fruit in the subsequent year. However there were some tendencies toward alternate bearing in individual trees, even though the same tendencies were not apparent at the orchard level. These data indicate that understanding yield behaviour of almond orchards is as much about understanding population dynamics of individual spurs and trees as it is about understanding the influence of general factors such as temperature, soil moisture, nutrient availability, etc., on overall orchard performance. This discovery could be of major significance for how future research concerning yield behaviour of almond orchards is conducted.

Acknowledgements:

Research Effort Recent Publications:

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Da Silva, D., Favreau, R., Auzmendi, I., and DeJong, T.M. 2011. Linking water stress effects on carbon partitioning by introducing a xylem circuit into L-PEACH. Annals of Botany (in press)

Lampinen, B.D., Tombesi, S., Metcalf, S. and DeJong, T.M. 2011. Spur behaviour in almond trees: relationships between previous year leaf area, fruit bearing and mortality. Tree Physiology (in press).

Negron, C., Contador, M.L., Lampinen, B.D., Metcalf, S.G., Guedon, Y., Costes, E., and DeJong, T.M. 2012. Using hidden semi-Markov chains to compare shoot structure of three different almond cultivars. Acta Horticulturae (in press).

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Lopez, G., Favreau, R.R., Smith, C., Costes, E., Prusinkiewicz, P. and DeJong, T.M. (2008) Integrating simulation of architectural development and source-sink behaviour of peach trees by incorporating Markov chain and physiological organ function sub-models into L-PEACH. Functional Plant Biology 35, 761-771.

Lampinen, B.D., Tombesi, S., Metcalf, S. and DeJong, T.M. 2011. Spur behaviour in almond trees: relationships between previous year leaf area, fruit bearing and mortality. Tree Physiology (in press).
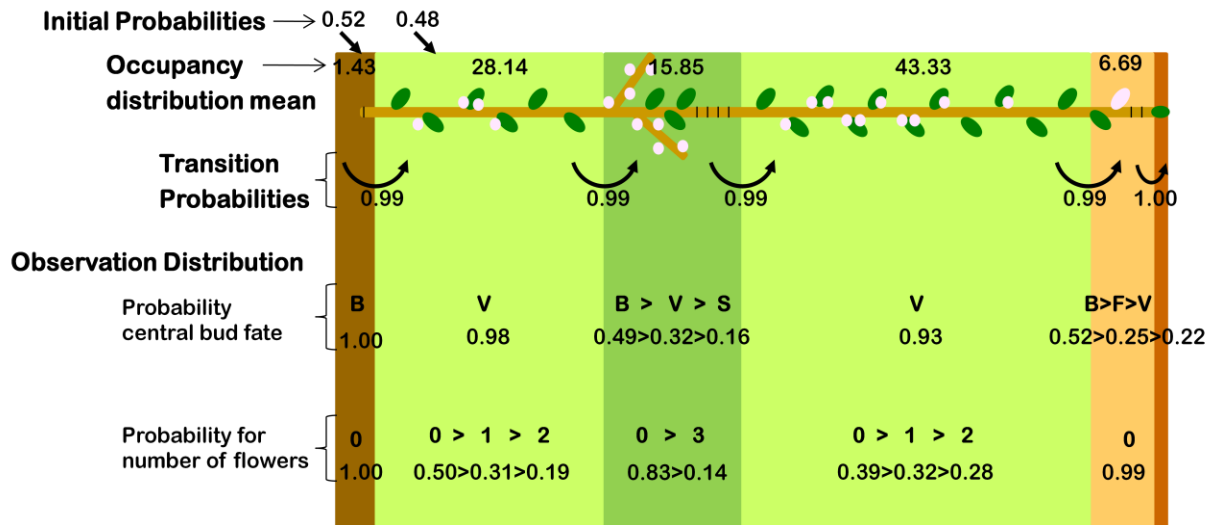
Figures:

Figure 1. Schematic representation of the hidden semi-Markov chain (HSMC) model for the long proleptic shoots of 'Nonpareil' with the four sets of estimated parameters. The mean length of the different zones of the shoots is represented by each segment. The observations for the central bud fate are: Blind node (B), Floral central bud (F), Vegetative bud (V), or Sylleptic shoot (S). The observations for number of flower buds per node are: no flower bud (0), 1 flower bud (1), 2 flower buds (2), and 3 or more flower buds (3).

Figure 2. An example of graphical computer output of the L-Peach model that demonstrates it's ability to simulate the accumulated influence of mild water stress over four seasons of growth. The only difference between the the control tree and the "drought" tree was that for the "drought" tree the simulated "irrigations" occurred every 3 weeks instead of every 3 days. Similar simulations will be possible with L-Almond when the new model is complete.
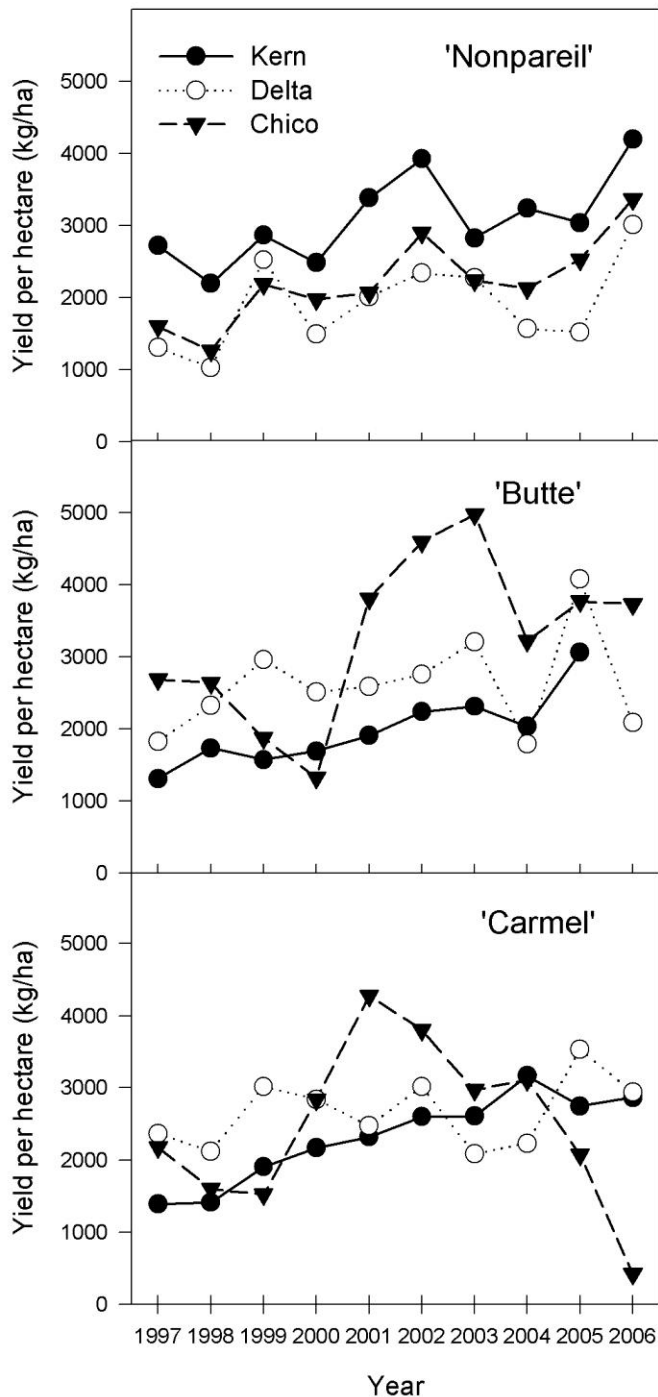
Figure 3. Yield trends from 1997 to 2006 in Chico, Delta and Kern orchards for 'Nonpareil', 'Butte' and 'Carmel' cultivars in the UC "regional variety trial". Trends toward alternate bearing were not readily apparent except for Nonpareil in Kern.
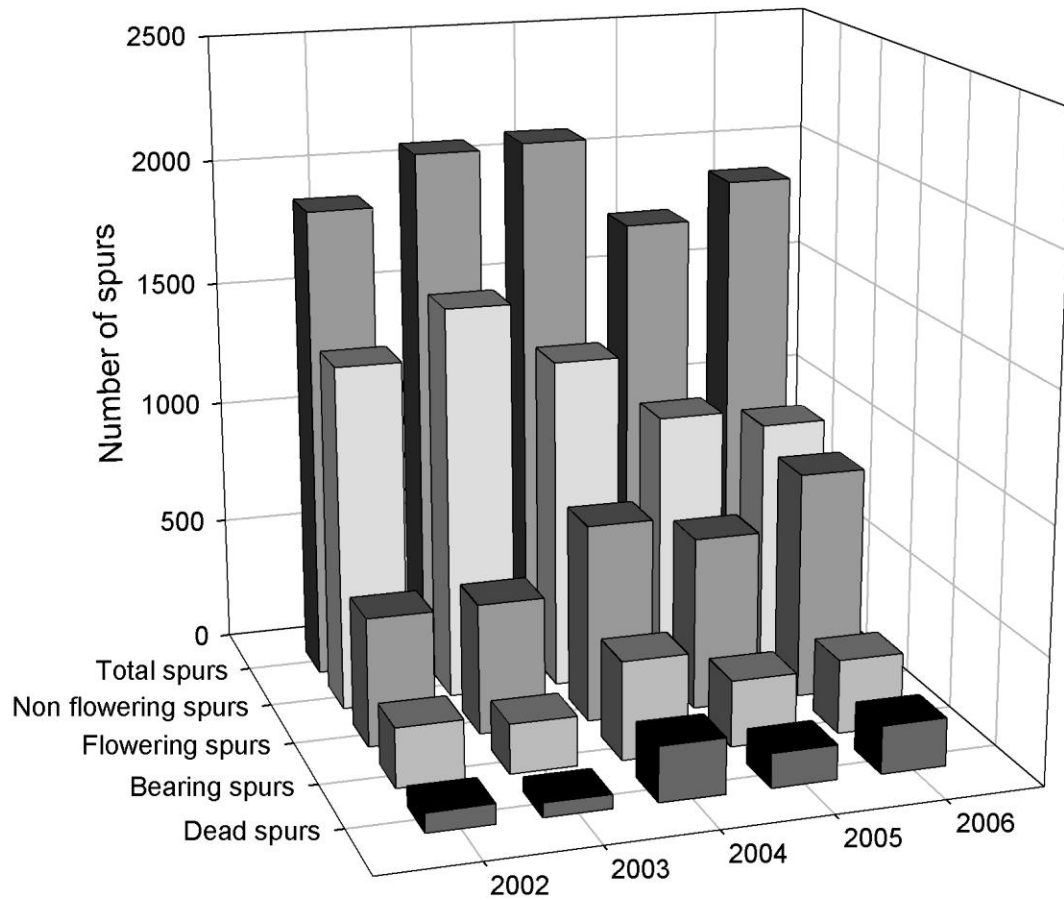
Figure 4. Spur population description over 5 years in the spur dynamics study in Kern County. Total number of the originally tagged spurs monitored and numbers of non flowering spurs, flowering spurs, bearing spurs and spurs dead in the year after bearing.