

How different pruning severities alter shoot structure: a modelling approach in young ‘Nonpareil’ almond trees

Claudia Negrón^A, Loreto Contador^A, Bruce D. Lampinen^A, Samuel G. Metcalf^A, Yann Guédon^B, Evelyne Costes^C and Theodore M. DeJong^{A,D}

^ADepartment of Plant Sciences, University of California, One Shields Avenue, Davis, CA 95616, USA.

^BUMR AGAP CIRAD/INRA/Montpellier SupAgro, Virtual Plants, INRIA, Avenue Agropolis, TA-A-108/03, 34398, Montpellier Cedex 5, France.

^CUMR AGAP CIRAD/INRA/Montpellier SupAgro, Equipe Architecture et Fonctionnement des Espèces Fruitières, Avenue Agropolis, TA-A-108/03, 34398 Montpellier Cedex 5, France.

^DCorresponding author. Email: tmdejong@ucdavis.edu

Abstract. Axillary meristem fate patterns along shoots, also referred to as shoot structure, appear to be fairly consistent among trees within a genotype growing under similar conditions. Less is known about shoot structural plasticity following external manipulations, such as pruning. The aim of this study on almond (*Prunus dulcis* (Mill.)) shoots was to investigate how pruning severity affects the structure of 1-year-old shoots that grew after pruning (regrowth shoots), the 2-year-old portion of shoots that remained from the previous year’s growth after pruning (pruned shoots), and whether regrowth shoots reiterate the structure of the original 1-year-old shoots before pruning. Three pruning severities were imposed and the structures along the different shoots were assessed by building hidden semi-Markov models of axillary meristem fates. The structures of regrowth and pruned shoots depended on pruning severity, but maintained some of the original shoot characteristics. Regrowth shoots developed more complex structures with severe pruning, but had simpler structures than original shoots indicating progressive simplification with tree age. Pruned shoot structures were affected by the severity of pruning, by the structure when the shoots were 1 year old, and probably by local competition among buds. Changes in structure due to pruning can be modelled and be predictable.

Additional keywords: axillary meristem, heading, hidden semi-Markov model, proleptic shoot, *Prunus dulcis*, sylleptic shoot.

Received 18 January 2014, accepted 29 October 2014, published online 26 November 2014

Introduction

The apical growth and axillary meristem fates of shoots are important because they largely determine final tree form, fruit bearing habit and cropping potential (Bernad and Socias 1998; Costes and Guédon 2002). They also determine the effectiveness of some horticultural operations such as pruning (Bernad and Socias 1998). Apical growth and axillary meristem fates are determined by the genetic background of trees, internal competition for resources and external limitations imposed by the environment. In the case of apical growth, which results from the initiation of new organs produced by the apical meristem (Barthélémy and Caraglio 2007), differences have been found among cultivars and the same cultivars growing on different rootstocks (Weibel *et al.* 2003), as well as among horticultural practices applied to different species (Millard and Neilsen 1989; Berman and DeJong 1997; Fumey *et al.* 2011). Axillary meristems (embryonic cells formed in the axils of the initiated leaf) may develop different fates (i.e. blind node, vegetative or flower buds, sylleptic shoots) as a shoot develops. Dormant axillary meristems that remain protected in vegetative buds

can develop proleptic shoots during the following growing season (Kervella *et al.* 1995; Barthélémy and Caraglio 2007). Axillary meristem fate during the first growing season has been linked to apical growth rate (Kervella *et al.* 1995) and is controlled by a complex set of biochemical signals from the shoot apex (apical dominance), other shoots and/or roots (Cline 1994; Cook *et al.* 2001; Dun *et al.* 2009). Outgrowth of lateral proleptic shoots during the second growing season is controlled by the terminal shoots and the lateral branches above the axillary meristem forming the shoot and has been called apical control (Wilson 2000). Differences in axillary meristem fate patterns along the shoots have been observed between cultivars of apple and almond (Costes and Guédon 2002; Gradziel *et al.* 2002), peach shoots of different lengths (Fournier *et al.* 1998), and apple shoots developed at different times during tree ontogeny (Renton *et al.* 2006). In addition, several studies have reported the effects of horticultural practices on the axillary meristem fate density per shoot in different species (Hipps *et al.* 1995; Guimond *et al.* 1998; Girona *et al.* 2005). However, less attention has been paid to the effects of cultural practices, such

as pruning, on the organisation of axillary meristem fates along shoots.

Many studies have reported that heading dormant shoots (pruning off the terminal ends of shoots) releases axillary meristems from apical control, inducing the outgrowth of lateral shoots on the 2-year-old pruned shoot (Barlow and Hancock 1960; Elfving 1990; Fumey *et al.* 2011). Subsequent lateral shoot growth is more vigorous than in unpruned trees (Maggs 1965; Mika 1976; Jonkers 1982; Fumey *et al.* 2011), leading to longer shoots as pruning severity increases (Jonkers 1982). However, the number of lateral shoots on 1-year-old regrowth shoots has been found to be similar to that of primary 1-year-old unpruned shoots (Fumey *et al.* 2011). This result supports the concept of 'reiteration' (Hallé *et al.* 1978), which was defined as the process whereby a new shoot repeats the basic components of the shoot from which it originated. This process is constrained by morphogenetic gradients due to tree ontogeny and branching order (Gatsuk *et al.* 1980; Barthélémy and Caraglio 2007). However, details of axillary meristem fate patterns of reiterated shoots in response to pruning severity have not been clearly documented.

Gradziel *et al.* (2002) qualitatively described the degrees and types of lateral branching along 1- and 2-year-old shoots in unpruned almond cultivars and seedling genotypes. However, in almond as in other species, some aspects of shoot characteristics can be modified with pruning (Asai *et al.* 1996a). Pruning is most important in the dormant period right after the first growing season because it determines the primary scaffolds that will constitute the main framework of an almond tree. Three first-year pruning methods are used depending on the objectives of the growers (emphasis on early production vs specific, uniform tree architecture), the growth habit of the cultivar and environmental conditions in the orchard. Short pruning consists of heading back each of the primary shoots to a length of 45–60 cm. This type of pruning promotes vigorous regrowth, facilitating the selection of secondary shoots for the development of scaffold branches. Long pruning consists of making small heading cuts at the ends of primary shoots. This pruning allows a more natural development of the branching habit and allows for more rapid fruit bearing. Intermediate pruning is a compromise between short and long pruning. This pruning induces less vigorous growth than short pruning but still promotes the small lateral branches that promote early fruiting, while making selection of scaffold branches more difficult than with short pruning (Asai *et al.* 1996a).

In recent years, stochastic models of axillary meristem fate have been developed based on qualitative botanical descriptions (Guédon *et al.* 2001). Hidden semi-Markov models (HSMMs) rely on the assumption that axillary meristem fate patterns along a shoot take the form of a succession of well differentiated homogeneous zones where the composition properties, in terms of axillary meristem fate, do not change substantially within each zone, but change markedly between zones. HSMMs have been useful for capturing the structured yet variable character of patterns observed along shoots in order to increase understanding of the processes that determine these patterns. (Guédon *et al.* 2001). This methodology has been used to represent the axillary meristem fate patterns along shoots of fruit trees under homogeneous horticultural practices

(Costes and Guédon 1997, 2002; Renton *et al.* 2006; Negrón *et al.* 2013). Quantification of the changes of these patterns due to different cultural practices, such as pruning, can also be investigated using this methodology.

This study entailed a quantitative analysis of the effects of three pruning severities on the patterns of axillary meristem fates along shoots that grow after pruning 2-year-old almond trees, using HSMMs. This study also aimed to determine whether the reiteration process after varying pruning severities produces 1-year-old shoots with axillary meristem fate patterns similar to those that previously existed on the pruned shoots from which they originated. Also, it is known that pruning stimulates lateral branching on the section of the shoot that remains after pruning but how the axillary meristem fate patterns of 1-year-old shoots interact with pruning severity to generate different axillary meristem fate patterns along 2-year-old shoots has not been previously described with HSMMs. In this study we also investigated how other features (e.g. length and node number) of regrowth and pruned shoots and their lateral shoots are affected by pruning severity. The pattern of axillary meristem fates along 1- and 2-year-old shoots is referred as the 'structure' of these shoots.

Materials and methods

Trial and field evaluations

This research was conducted in a commercial almond (*Prunus dulcis* (Mill.)) orchard planted in 2009 near Dixon (38°28'N, 121°45'W), in California's Central Valley. This study evaluated 'Nonpareil,' the main cultivar in California, grafted on 'Lovell' rootstock, after the first and second growing seasons after planting.

After the first growing season, forty 1-year-old shoots were selected on 16 trees (2–3 shoots per tree) in February, 2010. In this study, these shoots were called 'original shoots' (Fig. 1). The shoot lengths and the axillary meristem fates at every node from the base to the tip of each shoot were registered. Each node was categorised according to one of the following observations: blind node (axillary meristem fails to develop a bud), central vegetative bud (axillary vegetative meristem remains dormant), sylleptic shoot (shoot elongated from an axillary meristem immediately after initiation), or central floral bud (axillary meristem differentiated into a flower bud) (Kervella *et al.* 1995).

After the original shoot evaluation, three winter pruning treatments were imposed on 48 trees with 16 trees receiving one of the three pruning treatments. Pruning treatments were applied as described by Asai *et al.* (1996a) and based on the shoot length before pruning. The pruning treatments were: short pruning, in which shoots were cut back to about one-third their original length (thus, depending on their original lengths the part of the shoots remaining was between 45 and 56 cm long); intermediate pruning, in which the selected shoots were cut in half (the remaining length of the shoot was between 57 and 106 cm long); and long pruning, with small heading cuts, removing ~25 cm of the shoots (leaving shoots 104–141 cm long).

After the second growing season in February 2011, the 1-year-old shoots that grew from the vegetative bud proximal to the

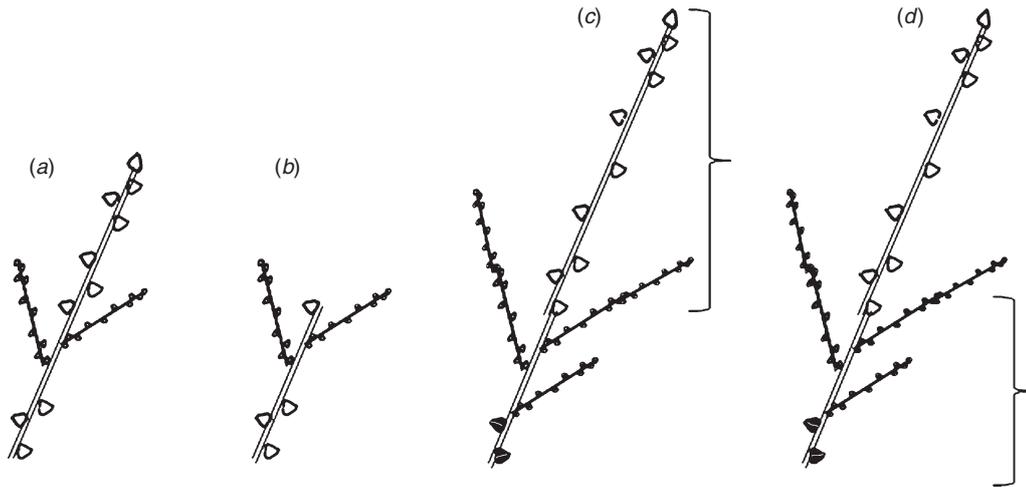


Fig. 1. Schematic representation of the type of shoots used in the study. Shoot types included: 1-year-old shoots that were initially selected for the study, or ‘original shoots’ (a), original shoots after pruning (b), 1-year-old shoots that grew from the vegetative bud proximal to the pruning cut after a dormant period, or ‘regrowth shoots’ (c, indicated with bracket), 2-year-old part of the original shoots left after pruning with development after a dormant period, or ‘pruned shoots’ (d, indicated with bracket).

pruning cuts were evaluated. In this study, these shoots are referred to as ‘regrowth shoots’ (Fig. 1). As described previously, the length and axillary meristem fates were assessed on 40 regrowth shoots per pruning treatment. Sylleptic shoot lengths and total number of flower buds per shoot were also registered. At the same time, axillary meristem fates on the 2-year-old part of shoots left after pruning were evaluated at each node from the base of the shoots up to the pruning cut. In this study, these shoots were called ‘pruned shoots’ (Fig. 1). Four axillary meristem fates could be observed at each node: blind nodes, proleptic shoots (shoots originating from axillary meristems after a dormant period), spurs (proleptic shoots with minimal elongation of the internodes) or sylleptic shoots (shoots that had grown the previous season). The lengths of the proleptic shoots that grew from axillary vegetative buds and the lengths of the proleptic shoots that grew from the terminal bud of previous year sylleptic shoots were also assessed.

Hidden semi-Markov model analyses

The pruning treatment effects on shoot structure, i.e. the patterns of axillary meristem fates along 1- and 2-year-old shoots were evaluated by building and interpreting the HSMMs of these shoot structures. The V-Plants software (release 0.9), part of the OpenAlea platform (Pradal *et al.* 2008), was used for analysis of the sequences of data collected along the shoots and for subsequent model building. For these analyses, the observations at each node were oriented from the base to the tip of shoots. Empirical intensity distributions that indicated the probability of each observation at each node rank were extracted from the sequences of data. These distributions indicated different zones along the shoots with homogeneous observations that were analysed with HSMMs. These models had four subsets of parameters that provided information about

the shoot structure: (i) initial probabilities, indicating the probability of occurrence of the first zone at the base of the shoots; (ii) transition probabilities, indicating the succession of zones along the shoots; (iii) occupancy distributions, indicating the length of each zone in terms of number of nodes; and (iv) observation distributions, indicating the probability of a specific axillary meristem fate within each zone. For further description of HSMMs constructed to describe shoots of fruit trees, see Costes and Guédon (1997), Costes and Guédon (2002) and Renton *et al.* (2006).

In the present study, HSMM parameters were estimated from assumptions established in different initial models and selecting an initial model which maximised the likelihood of the observed sequences for a given category of evaluated shoots using an iterative algorithm. These models relied on assumptions that were made from the intensity distributions regarding the number of homogeneous zones in the shoots, the succession of the zones, and the relative presence of types of axillary meristem fates in each zone. The succession of zones was modelled with a unidirectional (or ‘left-right’) Markov model, i.e. only transitions from one zone to the next were allowed, but transitions from a given zone to the previous ones were forbidden in all the evaluated shoots, except in the original shoots. Each axillary meristem fate was allowed in every zone, except in the first zone and last zone on 1-year-old shoots and in the first zone on 2-year-old shoots, where only blind nodes were observed. Theoretical distributions computed from the estimated model parameters and the empirical distributions extracted from the observed sequences were plotted together to evaluate estimated models (Guédon *et al.* 2001). This was done to ensure that the estimated models were not over-fitted.

To evaluate pruning treatment effects on axillary meristem fate of regrowth shoots without regard for structure, the frequency distribution of axillary meristem fates was extracted

from the observed sequences of each treatment. Since axillary meristem fate is a qualitative variable, a Chi-square test ($P < 0.05$) was used to determine if this variable was dependent on pruning. The number of nodes of pruned shoots was compared between shoots that had a hypothetical zone identified by the HSMM zone and shoots that lacked that zone using a t -test ($P < 0.05$). This helped to explain whether the lack of terminal zones was related to the number of nodes left after pruning.

Shoot features analyses

In this study the following shoot features were evaluated in the field or estimated from the field evaluations. The average internode length of regrowth shoots was estimated from their lengths and number of nodes. The number of lateral shoots and flower buds was normalised by the number of nodes per shoot, i.e. they are presented as proportion of the nodes present per shoot. The length and node number of the apical regrowth shoots and of pruned shoots were added to obtain the total length and node number of the primary scaffold. The mean length and number of all lateral shoots that grew on the regrowth shoots (sylleptic shoots) and on the pruned shoots (proleptic shoots that grew from axillary vegetative buds and from the sylleptic shoot terminal buds) were estimated from lateral shoot measurements. The three pruning treatments were compared on the basis of these shoot features. The homogeneity of variance and the normality of residuals were analysed first for each of the shoot features. When these assumptions of the ANOVA were met, Tukey's test ($P < 0.05$) was used for determining the mean separations (for length and internode length of apical regrowth shoots, sylleptic shoot length, number of proleptic shoots per node, and total number of nodes on apical regrowth and pruned shoots); otherwise, the

ANOVA by rank using the Kruskal–Wallis test and the associated Wilcoxon–Mann–Whitney tests ($P < 0.05$) for pairs of distributions were used (for node number of regrowth shoots, number of sylleptic shoots per node, number of flower buds per Wilcoxon–Mann–Whitney shoot and node, length of proleptic shoots, total length of apical regrowth plus pruned shoots, and length and number of lateral shoots on apical regrowth and pruned shoots). The latter statistical analyses were conducted with the Statistical Analysis Systems software (ver. 9.2, SAS Institute, Cary, NC, USA).

Results

Hidden semi-Markov model

One-year-old original shoot structure

Structure of 1-year-old shoots that grew during the first year after orchard planting corresponded to a 12-state HSMM (Fig. 2). The model had 10 consecutive zones (i.e. from zone i to zone $i+1$) that differed in the observation distributions and in the number of nodes. The model also had two reiterative alternating states, States 7 and 8, which corresponded to zones with similar composition and number of nodes that repeated towards the tip of the shoots. The last state of the model (Zone 11) corresponded to the terminal bud. Each zone was defined by an observation distribution of the axillary meristem fate: (i) Zone 0 at the base and Zone 10 at the tip of shoots had only blind nodes; (ii) Zones 1, 3, 5 and 8 had mainly vegetative buds and different zone lengths; (iii) Zones 2, 4 and 6 had mainly sylleptic shoots and different zone lengths; (iv) Zone 7 had blind nodes and a few vegetative buds; and (v) Zone 9 mainly contained central flower buds. The forward transition probabilities between consecutive zones were all high except those from Zone 8 to Zone 9. In this latter case, the most probable

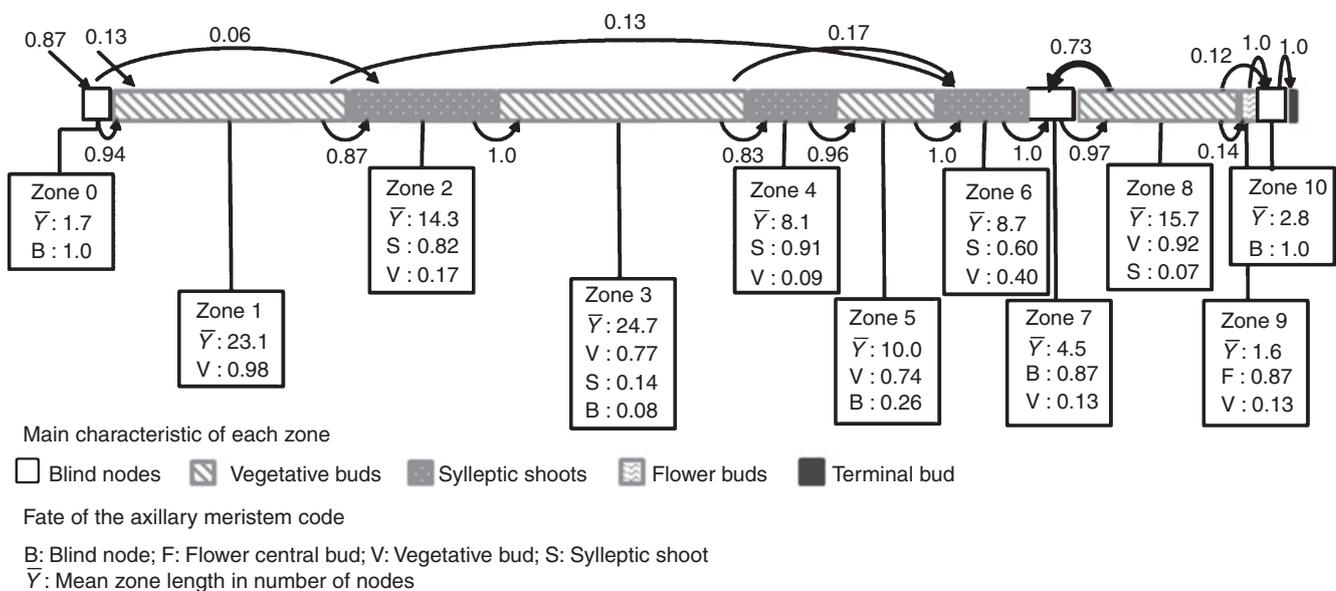


Fig. 2. Schematic representation of the 12-state hidden semi-Markov model for 1-year-old shoots in 1-year-old trees (original shoots). The zones of the shoots are represented by rectangles where the different fill-patterns indicate the main axillary production of the zone. The possible initial zones and transition between zones are shown by arrows and the attached probabilities are shown next to each arrow. The main axillary productions with the corresponding observation probabilities are indicated below for each zone. Diagram shows axillary productions that had an equal or greater than 0.05 probability.

transition leaving Zone 8 was the backward transition to Zone 7 corresponding to an alternation between Zones 7 and 8.

One-year-old regrowth shoot structure

Pruning treatments induced differences in the structure of regrowth shoots, but some patterns were also similar (Fig. 3). The regrowth shoot structure after the short pruning treatment was modelled with an 11-state HSMM (Fig. 3a). Zones were defined in the following way: (i) Zone 0 at the base and Zone 9 at the tip of the shoots had only blind nodes; (ii) Zone 1, 3, 5 and 7 had mainly vegetative buds differing in the observation probabilities and in the number of nodes; (iii) Zone 2, 4 and 6 corresponded to mainly sylleptic shoots also differing in the observation probabilities and in the node number; (iii) Zone 8 was a mixture of central flower buds with blind nodes and vegetative buds; and (iv) Zone 10 corresponded to the

terminal bud. In general, the zones with vegetative buds, except for Zone 7, were shorter and the zones with sylleptic shoots and flower buds were longer than the corresponding zones in the original shoots. This model had a high initial probability for Zone 0 and high forward transition probabilities between consecutive zones; therefore the zone succession was almost deterministic and most of the shoots were composed of 11 zones.

The structure of 1-year-old shoots that grew in response to the intermediate pruning treatments was modelled using a 9-state HSMM (Fig. 3b). Compositions of the three basal zones and four apical ones (formed before terminal bud zone) were similar to compositions of those zones in short-pruned regrowth shoots. Intermediate-pruned regrowth shoots had a long middle zone (Zone 3) composed of mainly vegetative buds and some sylleptic shoots, instead of having an additional middle zone with sylleptic shoots as observed in

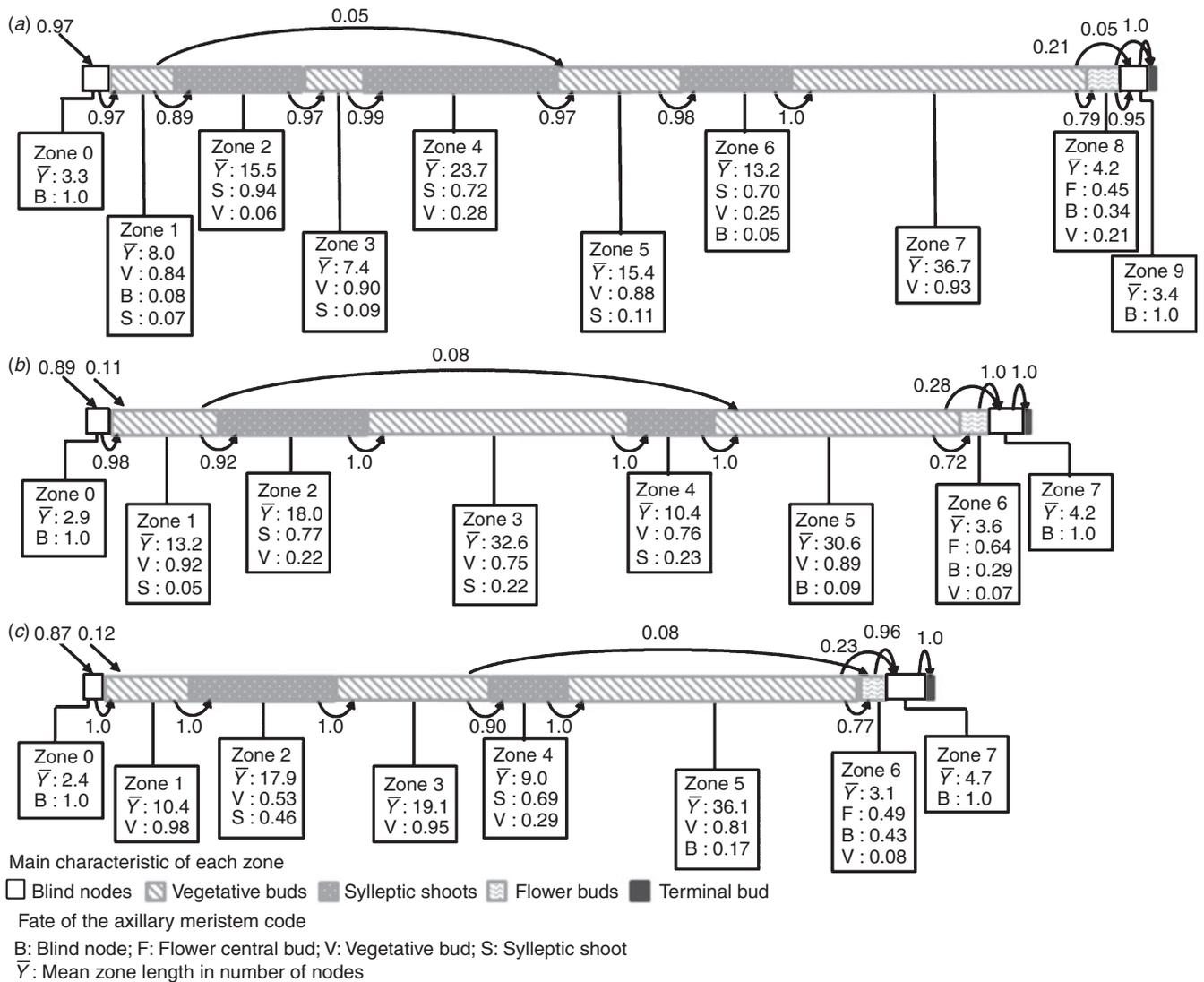


Fig. 3. Schematic representation of the hidden semi-Markov models for 1-year-old shoots that grew in response to the short (a), intermediate (b) and long (c) pruning treatments (regrowth shoots). Graphic components have the same meaning as in the model of Fig. 2. Diagram shows axillary productions that had an equal or greater than 0.05 probability.

short-pruned regrowth shoots (Zone 4). Zone succession was almost deterministic and all zones were present along most of the shoots (Fig. 3b).

The estimated HSMM for the structure of the shoots grown after the long pruning treatment also had nine states (Fig. 3c). The model was similar to the model for intermediate-pruned regrowth shoots. Both shoot types had the same number of zones and the zones were located in the same positions. Each zone had the same main axillary meristem fates, but sylleptic shoot probabilities were lower and blind node probabilities were higher in each zone of long-pruned regrowth shoots. Most of the zones had similar lengths as the same zone in intermediate-pruned regrowth shoots; however, Zone 3 was greatly reduced in long-pruned regrowth shoots.

The number of nodes having specific axillary meristem fates was affected by the pruning treatments ($P < 0.0001$; Fig. 4). The number of nodes that developed sylleptic shoots strongly increased with pruning severity. The large difference in the number of nodes with sylleptic shoots between treatments explained the variation in total number of nodes per shoot due to pruning (Table 1). Smaller differences in the numbers of blind nodes, vegetative buds and central flower buds were observed among treatments (Fig. 4).

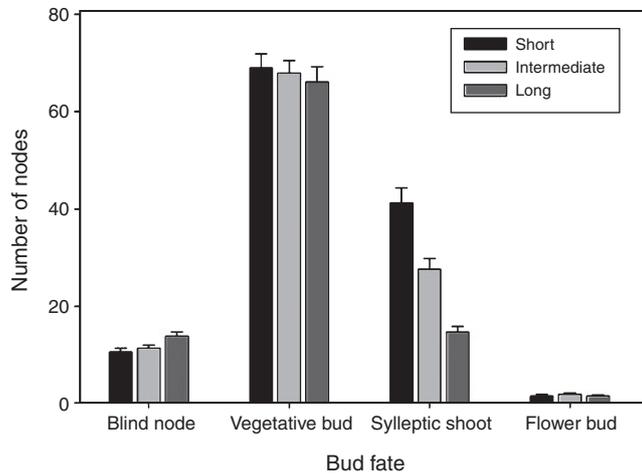


Fig. 4. Count/frequency distributions of the axillary meristem fate observations on 1-year-old shoots grown in response to short, intermediate or long pruning treatments. Significant differences were found in the number of axillary meristem fate observations according to the Chi-square test ($P < 0.05$).

Two-year-old pruned shoot structure

Pruning treatments also modified the structure of the remaining portion of the pruned shoots (Fig. 5). The estimated HSMM for the shoots that were more heavily pruned (short pruning treatment) had seven states (Fig. 5a). Each zone was defined by an observation distribution of the axillary meristem fate: (i) Zone 0 had only blind nodes; (ii) Zone 1 had mainly spurs; (iii) Zone 2 was composed of spurs and proleptic shoots; (iv) Zone 3 was a mixture of all observations, but predominately sylleptic shoots; (v) Zone 4 contained spurs, blind nodes and some proleptic shoots; and (vi) Zone 5 had mainly proleptic shoots, with some sylleptic shoots and blind nodes. Zone 6 represented the pruned part of the shoots. The zone composition of pruned shoots was defined by the composition of the zones when these shoots were 1 year old and by the pruning treatment. Zones 0 and 2 in original shoots (Fig. 2) were similar to Zones 0 and 3 in the short-pruned shoots (Fig. 5a). Zone 1 of original shoots (Fig. 2) became Zone 1 and Zone 2 in the pruned shoots (Fig. 5a). Similarly, Zone 3 of original shoots (Fig. 2) became Zone 4 and Zone 5 in the pruned shoots (Fig. 5a). Initial and transition probabilities indicated that Zones 0, 2 and 4 were not present in 41%, 5%, and 39% of shoots respectively (Fig. 5a). Shoots that did not have Zones 0 and 4 had fewer nodes than shoots that had those zones (data not shown). Presence of Zone 2 was not associated with number of nodes per shoot (data not shown).

The estimated HSMM for the structure of intermediate-pruned shoots had nine states (Fig. 5b). The first five zones were composed of similar axillary meristem fates as in short-pruned shoots (Fig. 5a). Subsequent zones were Zone 5, corresponding mainly to nodes with sylleptic shoots and some proleptic shoots; Zone 6, composed of spurs, blind nodes, and proleptic shoots; and Zone 7, which had mainly proleptic shoots, some sylleptic shoots, and blind nodes. The mean numbers of nodes of the first four zones were also similar to those in short-pruned shoots. In contrast, Zone 4 was longer in the intermediate-pruned shoots, since the pruning cut in the short-pruned shoots limited the length of this zone and induced the development of Zone 5, with proleptic shoots. The initial and transition probabilities indicated that Zones 0 and 2, as well as zones at the end of the shoots (4, 5, 6 and 7), were not present in some shoots (Fig. 5b). Shoots without one of these zones (except for Zones 4 and 5) had similar numbers of nodes as the shoots

Table 1. Effect of pruning treatments on the mean length, total number of nodes, internode lengths, length and number of sylleptic shoots, and number of flower buds per shoot and node of the apical 1-year-old regrowth shoots used for the structure evaluations. Significant differences between treatments are indicated by different letters ($P < 0.05$)

Treatment	Apical regrowth shoot						Sylleptic shoot				Flower buds			
	Length		Nodes		Internode length		Length		Number per node		Total number per regrowth shoot		Number per node	
	(cm)	s.d.	(no)	s.d.	(cm)	s.d.	(cm)	s.d.	(no)	s.d.	(no)	s.d.	(no)	s.d.
Short	193.8a	38.7	124.8a	21.4	1.53a	0.17	22.9a	5.7	0.31a	0.11	246.7a	122.2	1.94a	0.89
Intermediate	157.0b	39.4	110.6b	21.4	1.41b	0.16	17.6b	6.8	0.24b	0.09	196.1a	101.9	1.71a	0.74
Long	118.1c	32.0	96.7c	23.5	1.25c	0.17	13.2c	6.1	0.15c	0.05	114.1b	65.2	1.16b	0.55

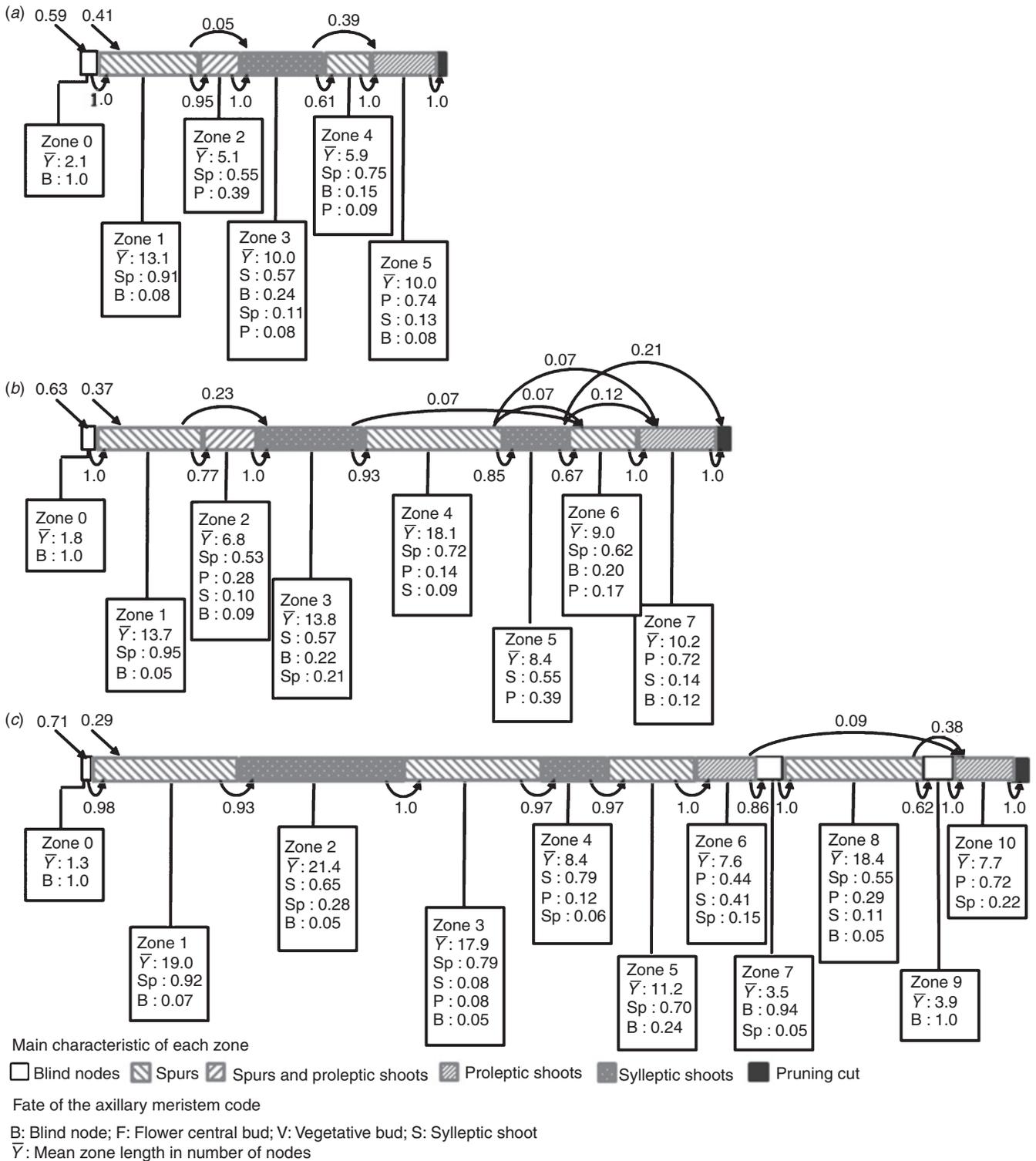


Fig. 5. Schematic representation of the hidden semi-Markov models of 2-year-old shoots after one season after being short (a), intermediate (b) or long pruned (c) (pruned shoots). Graphic components have the same meaning as in the model of Fig. 2. Diagram shows axillary productions that had an equal or greater than 0.05 probability.

that had these zones (data not shown). Shoots that lacked both Zones 4 and 5 had fewer nodes than shoots that had these zones (data not shown).

The estimated HSMM for long-pruned shoot structure had 12 states (Fig. 5c). The location and composition of the zones at the basal part of the long-pruned shoots was mainly

determined by the location and composition of the zones of these shoots when they were 1 year old (Fig. 2). Zones that had either blind nodes or sylleptic shoots in the original shoots corresponded to zones that had either blind nodes or sylleptic shoots in the pruned shoots. Zones composed of vegetative buds in the original shoots developed into zones that had spurs in the basal part of the pruned shoots and a mixture of spurs and proleptic shoots in the apical part of the pruned shoots (Figs 2, 5c). A model with two reiterative alternating zones, as used for analysing the original shoots, was not applicable to the long-pruned shoots. The reiterative vegetative bud zone in the original shoots (Zone 8) developed into a zone with a higher probability of proleptic shoots than spurs (Zone 10) close to the pruning cut and a zone with a higher probability of spurs than proleptic shoots (Zone 8) farther from the cut. The mean lengths of the zones on long-pruned shoots did not vary greatly in relation with lengths of corresponding zones in the previous year. The initial and transition probabilities indicated that most of the zones were present in all of the shoots, except for the basal blind node zone and the terminal zones (Fig. 5c). The number of nodes of shoots with or without Zones 0 and 7 were similar (data not shown). Only the shoots without Zone 9 had significantly fewer nodes than the shoots with that zone (data not shown).

Shoot features

The length of apical regrowth shoots used for structure evaluation significantly varied following pruning treatments ($P < 0.0001$; Table 1). Regrowth shoots observed in the short pruning treatment were the longest due to both more nodes per shoot ($P < 0.0001$) and longer internodes ($P < 0.0001$). Shoots grown after the long pruning treatment had fewer nodes and shorter internodes (Table 1). Short-pruned regrowth shoots were even longer than 1-year-old original shoots, with a mean length of 149.7 cm. Mean intermediate-pruned regrowth shoot length was very similar to the length of 1-year-old original shoots. However, on average the original shoots had more nodes (151.1) than all the regrowth shoots, and therefore shorter internodes (0.99 cm).

Mean sylleptic shoot length and the number of sylleptic shoots per node progressively decreased in the intermediate and long pruning treatments in comparison to the short pruning treatment ($P < 0.0001$; Table 1). Short- and intermediate-pruned regrowth shoots had more sylleptic shoots

per node than the original shoots (0.18 sylleptic shoots per node), which had more nodes than the long-pruned regrowth shoots. The number of flower buds was also influenced by pruning (Table 1). The long pruning treatment had significantly fewer flower buds per shoot and per node than the other two treatments ($P < 0.0001$).

The short pruning treatment induced the proleptic shoots to grow longer than those in the other treatments ($P < 0.0001$; Table 2). The mean number of proleptic shoots per node was also higher in the short pruning treatment than in the other two treatments ($P < 0.0001$; Table 2), mainly because fewer nodes remained after pruning in this treatment (data not shown). Pruning treatments did not affect the lengths of the proleptic shoots that developed from the terminal bud on sylleptic shoots produced the previous season ($P = 0.90$; Table 2).

The mean total lengths of apical regrowth shoots combined with the length of pruned shoots were similar among treatments ($P = 0.88$; Table 3). However, there were significant differences in the total number of nodes among treatments ($P < 0.0001$), with the long pruning treatments having more nodes (Table 3).

Lateral shoot production on regrowth and pruned shoots, resulted in significantly greater mean shoot lengths in the short pruning treatment ($P < 0.0001$; Table 3). However, the total number of lateral shoots was similar among treatments ($P = 0.95$; Table 3). The short pruning treatment also had more lateral shoots per node than the other treatments ($P = 0.001$; Table 3).

Table 2. Effect of pruning treatments on the mean length and number of proleptic shoots growing from axillary buds and on the length of proleptic shoots growing from sylleptic shoot terminal buds on 2-year-old pruned shoots

Significant differences between treatments are indicated by different letters ($P < 0.05$)

Pruning treatment	Proleptic shoots				Proleptic shoots on previous year sylleptic shoots	
	Length (cm)	s.d.	Number per node (no)	s.d.	Length (cm)	s.d.
Short	120.5a	26.5	0.26a	0.07	71.5a	22.2
Intermediate	73.3b	20.2	0.21b	0.07	72.0a	22.9
Long	59.8c	10.7	0.15c	0.05	68.1a	12.2

Table 3. Effect of pruning treatment on the total length and node number of apical regrowth shoot along with pruned shoot and on the mean length, total number and number per node of lateral shoots (i.e. sylleptic shoots on regrowth shoot and proleptic shoots on 2-year-old pruned shoots)

Significant differences between treatments are indicated by different letters ($P < 0.05$)

Pruning treatment	Apical regrowth and pruned shoot				Lateral shoots on regrowth and pruned shoot					
	Total length (cm)		Total number of nodes (no)		Mean length (cm)		Total number (no)		Total number per node (no)	
	s.d.	s.d.	s.d.	s.d.	s.d.	s.d.	s.d.	s.d.	s.d.	s.d.
Short	242.1a	39.2	167.2c	21.6	213.8a	31.2	57.5a	18.5	0.34a	0.09
Intermediate	240.0a	40.7	182.4b	22.1	162.9b	35.7	55.5a	13.4	0.30b	0.06
Long	244.7a	33.9	207.1a	26.0	141.1c	18.3	55.6a	10.7	0.26c	0.05

Discussion

Differences in pruning severity induced distinct responses in axillary meristem fate patterns along regrowth shoots and along pruned shoots. Differences were also found in the reiteration process which was studied by comparing the structures of the original shoot and the structures of the regrowth shoots after the pruning treatments. Additional features of the shoots were also modified by pruning treatments.

The structure of regrowth shoots showed similar organisations, but became more complex (i.e. more zones) as pruning severity increased (Fig. 3). More severe pruning also induced regrowth shoots that were longer and had more nodes than the regrowth shoots resulting from less severe pruning (Table 1), in agreement with previous studies (Barlow and Hancock 1960; Jonkers 1982; Marini and Barden 1982). The additional nodes stimulated by severe pruning mainly generated sylleptic shoots (Fig. 4) that were located in the middle part of the short-pruned regrowth shoots as is shown by the HSMM (Fig. 3a), creating a separate zone dominated by sylleptic shoots (Zone 4) in between two zones with vegetative buds (Zones 3 and 5). This was apparent because the zones on the basal part of the shoots (Zones 0, 1 and 2) in all treatments had similar lengths and compositions. In addition, Zones 6, 7, 8 and 9 in short-pruned regrowth shoots had similar lengths and compositions respectively as Zones 4, 5, 6 and 7 of the regrowth shoots in the other pruning treatments. Regrowth shoots in intermediate and long pruning treatments did not develop the additional zone with sylleptic shoots (Zone 4) as in short-pruned regrowth shoots; instead they only developed a single zone with mostly vegetative buds (Zone 3). In long-pruned regrowth shoots, Zone 3 (the vegetative bud zone) was the only zone with greatly decreased length compared with the intermediate-pruned regrowth shoots.

Fournier *et al.* (1998) and Renton *et al.* (2006) also reported a progressive increase in shoot structural complexity with the length of shoots in peach and apple, respectively. They found that a zone with sylleptic shoots developed in the middle part of longer shoots that was not observed in shorter shoots. Therefore, they suggested that the succession of zones along shoots is defined while the shoots are growing. For instance, faster growth rates have been related to a release of apical dominance with consequent outgrowth of sylleptic shoots (Génard *et al.* 1994). On the other hand, the development of vegetative and flower buds have been associated with slower shoot growth rates than for the development of sylleptic shoots (Kervella *et al.* 1995), and flower buds are formed when the plastochron is shorter than the plastochron associated with vegetative buds (Fulford 1966). Blind nodes are associated with even lower shoot growth rates than vegetative and flower buds (Kervella *et al.* 1995).

The growth rates of regrowth shoots can be modified by pruning (Mika 1986; Li *et al.* 1994; Tworcoski *et al.* 2006), and tend to increase with pruning severity (Mika 1986; Li *et al.* 1994). In this study, short pruning apparently induced a higher growth rate in regrowth shoots, which in turn promoted development of an extra sylleptic shoot zone (Zone 4) as well as more and longer sylleptic shoots than in the regrowth shoots of other treatments. Correspondingly, intermediate pruning may have induced higher growth rates than long

pruning, since higher probabilities of longer sylleptic shoots and lower probabilities of blind nodes were observed in all the zones of the intermediate-pruned regrowth shoots compared with the long pruned shoots.

Replacement of shoots with shoots having similar structure as an original shoot after shoot breakage or removal by pruning has been termed 'traumatic reiteration' (Barthélémy and Caraglio 2007). The evaluation of the reiteration process in this study indicated that different pruning severities induced regrowth shoots that had simpler structures (i.e. had fewer zones) than the original shoots (Figs 2, 3). However, short pruning provoked regrowth shoots that had more similarities to original shoots than the regrowth shoots of lesser pruning severity treatments. Short-pruned regrowth shoots had seven basal zones with compositions similar to the equivalent zones of original shoots, but the zones with sylleptic shoots were longer in short-pruned regrowth shoots than the original shoots. Similarities between the structure of the original shoots and the regrowth shoots produced subsequent to short pruning may have occurred because both shoots grew in response to severe dormant season pruning that promoted vigorous vegetative growth. The largest differences between these shoots were found in the apical zones. Towards the shoot apex, original shoots developed vegetative bud zones interrupted by blind node zones, while regrowth shoots developed only one vegetative bud zone despite their vigorous vegetative growth (i.e. longer shoots). The simplification of structure on the apical part of the short-pruned regrowth shoots (i.e. lack of reiterative alternating vegetative bud and blind node zones) might be explained by an earlier maturation of the meristems due to their ontogenetic age and greater distances from the roots to the meristems that originated the regrowth shoots (Sachs 1999; Renton *et al.* 2006). The even lower number of zones in the lighter pruning treatments indicated that less shoot vigour allowed for progressive shoot structure simplification. This is apparently a natural process that occurs with tree age if pruning and limb breakage does not occur (Renton *et al.* 2006).

Structure of 2-year-old pruned shoots depended on pruning severity and the structure established when the shoots originally developed (Figs 2, 5). In general, 2-year-old shoots in all pruning treatments developed proleptic shoots in every zone, except for the two basal zones in short- and intermediate-pruned shoots, and the three basal zones in long-pruned shoots. The lack of proleptic shoots in these basal zones comports with the concept of an acrotonic gradient being restored after pruning (Barlow and Hancock 1960; Mika 1986; Fumey *et al.* 2011). Thus the proleptic shoot lengths decreased from the apical to the basal ends of the shoots, and therefore only spurs developed from the vegetative buds located in the basal zones. The lack of certain zones in the apical part of the shoots was related to fewer nodes per shoot being left after pruning, and as a consequence, to the zone of the original shoot where the cut was made. Thus, when fewer nodes were left after pruning in a shoot, a zone in the original shoot could have been removed completely, and therefore the zone in the pruned shoots was not present. The composition of distal zones of intermediate- and short-pruned shoots mainly consisted of proleptic shoots growing from vegetative buds that were stimulated to grow due to the removal of apical control (Wilson 2000) and of other axillary meristem fates established

the previous year, i.e. sylleptic shoots or blind nodes. Only long pruning produced proleptic shoots and spurs in the zone just below the pruning cut. In this distal zone of long-pruned shoots, and in other zones along the shoots in all the pruning treatments, the development of either proleptic shoots or spurs was probably determined by local competition of vegetative buds for assimilates during budburst and by inhibitory effects exerted by shoot apices (Oliveira and Priestley 1988; Costes and Guédon 2002; Fumey *et al.* 2011).

Pruning also affected the principal features of regrowth and pruned shoots as well as of their lateral shoots. The increase of the apical regrowth shoot length associated with pruning severity was related to both an increased number of nodes and greater internode lengths (Table 1). As previously reported more severe pruning also produced the outgrowth of more and longer sylleptic shoots from the regrowth shoots as well as of more proleptic shoots from pruned shoots (Barlow and Hancock 1960; Jonkers 1982; Marini and Barden 1982) (Table 2). Pruning has been reported to promote proleptic branching by releasing vegetative buds of inhibitory growth effects exerted by the shoot apex. This is thought to be moderated by a complex of hormones, including auxin, cytokinin and strigolactone (Cline 1994; Cook *et al.* 2001; Dun *et al.* 2009). Pruning is also believed to produce an imbalance between roots and shoots, therefore an increase of the number and length of lateral shoots with pruning severity may have resulted in increased assimilate distribution towards shoots that served to restore the root-shoot balance (Marini and Barden 1982; Génard *et al.* 2008). This may explain the similar total shoots lengths (combined lengths of pruned and of the apical regrowth shoots) attained among treatments after the end of the second growing season (Table 3). Restoration of balance after pruning could be also achieved by the outgrowth of equal numbers of lateral shoots (i.e. proleptic shoots in the pruned shoots and sylleptic shoots on the regrowth shoots) among treatments to compensate for the lateral sylleptic shoots removed by pruning. Thus, the differences in shoot structures may be a re-equilibration of the total number of laterals. This re-equilibration of the number of lateral has been previously reported in other species (Marini 2002; Fumey *et al.* 2011).

Less severe pruning produced fewer flower buds per shoot and per node in the regrowth shoots (Table 3). This observation runs contrary to the results of other studies reporting that winter pruning decreases flower bud formation (Mika 1976; Mika *et al.* 1983). However, since 'Nonpareil' almond produces flower buds on both spurs and long shoots (Asai *et al.* 1996b), the increase of sylleptic shoots on regrowth shoots with pruning severity in this study conferred additional sites for flower bud formation and flower numbers in the second year actually increased with pruning severity. In addition, the number of flower buds was not significantly different between intermediate and short pruning treatments, indicating that pruning severity was not always associated with increased flower bud formation even though it did generate more sylleptic shoots with the ability to develop flower buds.

Although general shoot growth vigour responses of trees to pruning have been described and known for many years (Mika 1986), details about shoot structures that develop subsequent to pruning are less understood. This study provides clearer

understanding of those structures and the factors that likely influence them. The models used in this study provided a characterisation and a quantitative description of shoot structures. Because these models are purely phenomenological, the biological determinates for such structuring need further investigation before more mechanistic modelling approaches can be developed. Nevertheless, they indicate that axillary meristem fate along the shoot is not random and is organised in distinctive zones along the shoots. This structure is modified with pruning severity. The evaluation of shoot structures using the models such as those described in this research can be useful for predicting the structure of the regrowth and pruned shoots in response to pruning severity and thus when making pruning decisions in an orchard. Even though the precise shoot structures may differ by cultivar or horticultural practices, similar general responses to pruning are likely. Regardless of the model complexity, future research using these models can be done to investigate the effect of other horticultural practices in shoot structure. In addition, they can be integrated into more complex models of whole tree architecture using their capability to predict branching as trees age.

Acknowledgements

We thank the grower, Ron Timothy, for providing the study site and farm advisor Carolyn DeBuse for logistic support and study set up.

References

- Asai WK, Edstrom JP, Connell JH (1996a) Training young trees. In 'Almond production manual.' (Ed. WC Micke) pp. 121–124. (University of California: Oakland)
- Asai WK, Micke WC, Kester DE, Rough D (1996b) The evaluation and selection of current varieties. In 'Almond production manual.' (Ed. WC Micke) pp. 52–60. (University of California: Oakland)
- Barlow HWB, Hancock CR (1960) The experimental modification of branch form in an apple rootstock. *Botanical Gazette* **121**, 208–215. doi:10.1086/336071
- Barthélémy D, Caraglio Y (2007) Plant architecture: a dynamic, multilevel and comprehensive approach to plant form, structure and ontogeny. *Annals of Botany* **99**, 375–407. doi:10.1093/aob/mcl260
- Berman ME, DeJong TM (1997) Diurnal patterns of stem extension growth in peach (*Prunus persica*): temperature and fluctuations in water status determine growth rate. *Physiologia Plantarum* **100**, 361–370. doi:10.1111/j.1399-3054.1997.tb04794.x
- Bernad D, Socias R (1998) Bud density and shoot morphology of some self-compatible almond selections. *Acta Horticulturae* **470**, 273–279.
- Cline MG (1994) The role of hormones in apical dominance. New approaches to an old problem in plant development. *Physiologia Plantarum* **90**, 230–237. doi:10.1111/j.1399-3054.1994.tb02216.x
- Cook NC, Bellstedt DU, Jacobs G (2001) Endogenous cytokinin distribution patterns at budburst in Granny Smith and Braeburn apple shoots in relation to bud growth. *Scientia Horticulturae* **87**, 53–63. doi:10.1016/S0304-4238(00)00161-8
- Costes E, Guédon Y (1997) Modeling the sylleptic branching on one-year-old trunks of apple cultivars. *Journal of the American Society for Horticultural Science* **122**, 53–62.
- Costes E, Guédon Y (2002) Modelling branching patterns on 1-year-old trunks of six apple cultivars. *Annals of Botany* **89**, 513–524. doi:10.1093/aob/mcf078
- Dun EA, Brewer PB, Beveridge CA (2009) Strigolactones: discovery of the elusive shoot branching hormone. *Trends in Plant Science* **14**, 364–372. doi:10.1016/j.tplants.2009.04.003

- Elfving DC (1990) Growth and productivity of 'Empire' apple trees following a single heading-back pruning treatment. *HortScience* **25**, 908–910.
- Fournier D, Costes E, Guédon Y (1998) A comparison of different fruiting shoot of peach tree. *Acta Horticulturae* **465**, 557–566.
- Fulford RM (1966) The morphogenesis of apple buds. III. The inception of flowers. *Annals of Botany* **30**, 207–219.
- Fumey D, Lauri P-É, Guédon Y, Godin C, Costes E (2011) How young trees cope with removal of whole or parts of shoots: an analysis of local and distant responses to pruning in 1-year-old apple (*Malus × domestica*; Rosaceae) trees. *American Journal of Botany* **98**, 1737–1751. doi:10.3732/ajb.1000231
- Gatsuk LE, Smirnova OV, Vorontzova LI, Zaigolnova LB, Zhukova LA (1980) Age states of plants of various growth forms: a review. *Journal of Ecology* **68**, 675–696. doi:10.2307/2259429
- Génard M, Pagès L, Kervella J (1994) Relationship between sylleptic branching and components of parent shoot development in the peach tree. *Annals of Botany* **74**, 465–470. doi:10.1006/anbo.1994.1142
- Génard M, Dauzat J, Franck N, Lescouret F, Moitrier N, Vaast P, Vercambre G (2008) Carbon allocation in fruit trees: from theory to modelling. *Trees* **22**, 269–282. doi:10.1007/s00468-007-0176-5
- Girona J, Gelly M, Mata M, Arbones A, Rufat J, Marsal J (2005) Peach tree response to single and combined deficit irrigation regimes in deep soils. *Agricultural Water Management* **72**, 97–108. doi:10.1016/j.agwat.2004.09.011
- Gradziel TM, Kester DE, Martinez-Gomez P (2002) A development based classification for branch architecture in almond. *Journal American Pomological Society* **56**, 106–112.
- Guédon Y, Barthélémy D, Caraglio Y, Costes E (2001) Pattern analysis in branching and axillary flowering sequences. *Journal of Theoretical Biology* **212**, 481–520. doi:10.1006/jtbi.2001.2392
- Guimond CM, Lang GA, Andrews PK (1998) Timing and severity of summer pruning affects flower initiation and shoot regrowth in sweet cherry. *HortScience* **33**, 647–649.
- Hallé F, Oldeman RAA, Tomlinson PB (1978) Opportunistic tree architecture. In 'Tropical trees and forests: an architectural analysis'. pp. 269–331. (Springer-Verlag: Berlin)
- Hipps NA, Pagès L, Huguet JG, Serra V (1995) Influence of controlled water supply on shoot and root development of young peach trees. *Tree Physiology* **15**, 95–103. doi:10.1093/treephys/15.2.95
- Jonkers H (1982) Testing Koopmann's rules of apple tree pruning. *Scientia Horticulturae* **16**, 209–215. doi:10.1016/0304-4238(82)90069-3
- Kervella J, Pagès L, Génard M (1995) Growth context and fate of axillary meristems of young peach trees: influence of parent shoot growth characteristics and of emergence date. *Annals of Botany* **76**, 559–567. doi:10.1006/anbo.1995.1133
- Li S-H, Zhang X-P, Meng Z-Q, Wang X (1994) Responses of peach trees to modified pruning: 1. Vegetative growth. *New Zealand Journal of Crop and Horticultural Science* **22**, 401–409. doi:10.1080/01140671.1994.9513852
- Maggs DH (1965) Dormant and summer pruning compared by pruning young apple trees once on a succession of dates. *Journal of Horticultural Science* **49**, 249–265.
- Marini RP (2002) 'Pruning peach trees.' (Virginia Cooperative Extension: Blacksburg, VA, USA)
- Marini RP, Barden JA (1982) Growth and flowering of vigorous apple (*Malus domestica*) trees as affected by summer or dormant pruning. *Journal of the American Society for Horticultural Science* **107**, 34–39.
- Mika A (1976) Growth intensity and flower bud formation in young vegetatively propagated apple trees. *Acta Horticulturae* **56**, 195–200.
- Mika A (1986) Physiological responses of fruit trees to pruning. In 'Horticultural reviews. Vol. 8'. (Ed. J Janick) pp. 337–378. (John Wiley & Sons Inc.: Hoboken, NJ, USA)
- Mika A, Grochowska MJ, Karaszewska A, Williams MW (1983) Effects of dormant and summer pruning disbudding and growth retardants on growth flower bud formation and fruiting of young apple (*Malus domestica*) trees. *Journal of the American Society for Horticultural Science* **108**, 655–660.
- Millard P, Neilsen GH (1989) The influence of nitrogen supply on the uptake and remobilization of stored N for the seasonal growth of apple trees. *Annals of Botany* **63**, 301–309.
- Negrón C, Contador L, Lampinen BD, Metcalf SG, DeJong TM, Guédon Y, Costes E (2013) Systematic analysis of branching patterns of three almond cultivars with different tree architectures. *Journal of the American Society for Horticultural Science* **138**, 407–415.
- Oliveira CM, Priestley CA (1988) Carbohydrate reserves in deciduous fruit trees. In 'Horticultural reviews. Vol. 10'. (Ed. J Janick) pp. 403–430. (John Wiley & Sons Inc.: Hoboken, NJ, USA)
- Pradal C, Dufour-Kowalski S, Boudon F, Fournier C, Godin C (2008) OpenAlea: a visual programming and component-based software platform for plant modelling. *Functional Plant Biology* **35**, 751–760. doi:10.1071/FP08084
- Renton M, Guédon Y, Godin C, Costes E (2006) Similarities and gradients in growth unit branching patterns during ontogeny in 'Fuji' apple trees: a stochastic approach. *Journal of Experimental Botany* **57**, 3131–3143. doi:10.1093/jxb/erl075
- Sachs T (1999) 'Node counting': an internal control of balanced vegetative and reproductive development. *Plant, Cell & Environment* **22**, 757–766. doi:10.1046/j.1365-3040.1999.00220.x
- Twoorkoski T, Miller S, Scorza R (2006) Relationship of pruning and growth morphology with hormone ratios in shoots of pillar and standard peach trees. *Journal of Plant Growth Regulation* **25**, 145–155. doi:10.1007/s00344-005-0123-x
- Weibel A, Johnson RS, DeJong TM (2003) Comparative vegetative growth responses of two peach cultivars grown on size-controlling versus standard rootstocks. *Journal of the American Society for Horticultural Science* **128**, 463–471.
- Wilson BF (2000) Apical control of branch growth and angle in woody plants. *American Journal of Botany* **87**, 601–607. doi:10.2307/2656846