States of Water in Summer-dormant Apple Buds Determined by Proton Magnetic Resonance Imaging

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Abstract. Magnetic resonance imaging was used to determine water states in paradormant apple (*Malus domestica* Borkh.) buds and during early events when buds resumed growth. Proton density and states of water were determined by creating image maps of proton density and relaxation times (T₂). Summer-dormant (paradormant) buds had T₂ relaxation times up to 30 ms. This water in bud tissues is considered relatively free compared to water that had T₂ relaxation times of <1 ms in other parts of the stem and bark. Buds were forced to grow either by pruning off the terminal bud or by starting the bud with thidiazuron (TDZ). Both treatments gave essentially the same results. After treatment, buds started to grow immediately and water moved into the stem and into the bud. As there was more free water in the bud, T₂ values ranged up to 50 ms. There appeared to be an inhibitory gradient down on the shoot, which was removed temporarily by excising the top bud. However, between the 2nd and 10th day after removal of the top bud this dominance was reinstated by the highest bud on the stem, which eventually formed a shoot. TDZ treatment overcame this inhibitory gradient effect. There was also a growth potential gradient coinciding with the inhibitory gradient. The growth of lower buds was much slower than that of the upper buds. The growth potential gradient was not overcome by TDZ treatments.

Magnetic resonance imaging (MRI) is a relatively recent method for determining nondestructively the states of water in biological tissues, including plants. MRI was used for creating images of specific tissues in apple buds (Faust et al., 1991), and in apple (Wang et al., 1988) and pear (*Pyrus communis* Linn) fruit (Wang and Wang, 1989) and *Pelargonium* roots (Brown et al., 1986). Water states are determined usually by determining T₂ relaxation times. In our earlier studies, we created images of T₂ times. In more recent studies (Millard et al., 1993), we created T₂ images that associate states of water with tissues within the structure.

Dormancy in deciduous fruit trees and other temperate-zone woody perennials is defined as cessation of observable growth. This phenomenon is different during the winter and summer and is termed endodormancy and paradormancy (Lang, 1987), respectively. Different mechanisms are believed to control each of the two dormancies (Hillman, 1984; Saure, 1985).

Hillman (1984) summarized 14 treatments that promote growth of lateral buds in a wide variety of plants. In paradormancy, shoot tips and/or adjacent leaves control the outgrowth of lateral buds (Faust, 1989; Saure, 1985). Because of a high degree of interdependence between the shoot tip and lateral buds, paradormancy can be classified as correlative inhibition. Decapitation (excision of the stem apex) is the most effective means of removing paradormancy and allowing the shoots to grow (Faust, 1989). Treatment with cytokinin analogs also removes dormancy effectively in apple (Broome and Zimmerman, 1976; Wang et al., 1986). The correlative inhibition on the current-year shoot is potentially an excellent experimental model for the study of spatial organization of developmental activities in plants (Phillips, 1975).

In most species, the inhibited lateral buds in ascending position along the shoot respond to the activation differently. Suzuki (1990) demonstrated that there is an inhibitory gradient along the shoot in mulberry (*Morus alba* Linn.), which, in general, McIntyre (1977) ascribed to the accumulation of nutrients in water. The observation that bud growth of some species is prompted by water availability and high humidity also suggests that water may play a critical role in paradormancy. However, without short-term monitoring of changes in various states of water we can not obtain an in-depth analysis of the involvement of water in budbreak. We report on our attempt to evaluate the states of water in dormant and activated summer buds if apple.

Materials and Methods

Shoots of apple cultivar Anna, grown in the experimental orchard of the Beltsville Agricultural Research Center, Beltsville, Md., were used for the experiments. After terminal bud was set and shoot growth had stopped (early July), shoots of uniform length (16 buds long) were selected and four treatments were applied to these shoots. Pruning treatments were modified from Gyuro (1980) based on pruning severity and were applied to shoots as follows: 1) light pruning, only the terminal bud was removed, leaving a "long" shoot (cut was made below bud 1); 2) medium pruning, 25% of the shoot was removed, leaving a "medium"-length shoot (cut was made below bud 4); 3) severe pruning, 75% of the shoot was removed, leaving a "short" shoot (cut was made below bud 13); and 4) thidiazuron (TDZ) treatment (100 µM) was applied to all buds without pruning. Lateral buds sprouted in about 10 days after pruning or TDZ treatment. Samples were taken for MRI before pruning, 48 and 240 h after pruning or 168 h after treatment with TDZ. Five consecutive buds from the top of each shoot were imaged, with the exception of short shoots, on which only three buds remained after pruning. Each treatment consisted of 12 shoots, three of which were imaged.

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Fig. 1. Proton density and T2 maps of paradormant apple buds before and 48 h after activation by removal of the terminal bud. (a) Proton density map of bud 2 before treatment. (b) Proton density map of bud 2, 48 h after activation. (c) T2 image of bud 2 before treatment. (d) T2 image of bud 2, 48 h after activation. (e) Proton density image of bud 5 before treatment. (f) Proton density image of bud 5, 48 h after activation. (g) T2 image of bud 5 before treatment. (h) T2 image of bud 5, 48 h after treatment. T2 values are colored in unequal steps up to 100 ms: dark red, <10; red, 11–15; yellow, 16–20; green, 26–30; maroon, 31–35; pink, 36–50; and purple, 51–100 ms. Proton spin density (bars) is expressed as an arbitrary equally spaced scale from 1 to 8, from pink (low) to white (high). Longer relaxation time means more free water; higher color in proton spin density means more total water. Disregard T2 values where pink on the proton density image coincides with purple on the T2 image. B = bud; S = stem; and M = meristematic tissues.

Table 1. Effect of pruning treatments and thidiazuron on budbreak of apple shoots.

Bud position ²	Shoot length following pruning			TDZ-treated
	Long	Medium	Short	
1	Cut			96
2	100	---	---	96
3	87	---	---	94
4	9	Cut	---	92
5	0	67	---	92
6	0	5	---	90
7	0	0	---	90
8	0	0	---	90
9	0	0	---	89
10	0	0	---	89
11	0	0	---	88
12	0	0	---	87
13	0	0	Cut	---
14	0	0	100	---
15	0	0	0	---
16	0	0	0	---

²Shoots were pruned distal to the bud position indicated.

For MRI imaging, shoots were placed into 1-mm NMR tubes (Faust et al., 1991). Image slices were taken perpendicular to the axis of the shoot through the midpoint of buds (Millard et al., 1993). For imaging, a Bruker MSL 400 MHz (9.4-T) instrument was used (Bruker Instrument Co., Manning Park, Bellerica, Mass.). Maps were created with 256 × 256 picture elements (pels), corresponding to voxels of the tissue. Proton density and T2 relaxation signals were determined pel-by-pel. Methodology used was as described in Millard et al. (1993). Images were highly replicable; but, because of the large number of pels per image, statistical calculations were not possible.

Images were produced in color, which is essential to express quantitative values visually in an image. A different color is assigned to a range of T2 times; thus, a map is created associating a given T2 range to a specific area within the tissue. T2 values are colored in unequal steps up to 100 ms: dark red, <10; red, 11-15; yellow, 16-20; green, 21-30; maroon, 31-35; pink, 36-50; and purple, 51-100 ms. Proton spin density is expressed as an arbitrary equally spaced scale from 1 to 8, from pink (low) to white (high). Longer relaxation time means more free water; higher color in proton spin density means more water.

Fig. 2. Proton density and T2 maps of buds 5 and 8, 48 h after activation by removal of the top portion of the shoot (four buds). (a) Proton density image of bud 5. (b) Proton density image of bud 8. (c) T2 image of bud 5. (d) T2 image of bud 8. Explanation for colors and letters are the same as in Fig. 1.

In some images where proton density signals were very low, the T2 times do not represent a valid region. Therefore, in images where low proton spin density (pink) was associated with high T2 values, these values must be disregarded, as discussed by Millard et al. (1993).

Results

MRI proton density maps and T2 images of buds 2 and 5, before the treatment started, are illustrated in Fig. 1. Judging from proton

density maps, water content of both buds was low, registering between 1 and 2 on the relative scale of 1 to 8. Bud 2 contained slightly more water (Fig. 1a) than bud 5 (Fig. 1e).

Removing the apical bud, or heading the shoot at any place, usually induced breaking of one or two buds immediately below the pruning cut, with the lower buds apparently remaining dormant (Table 1). Responding buds usually became swollen and broke around 10 days after heading. MRI revealed that, before pruning, bud 5 was less developed than bud 2 (Fig. 1 a and c vs. e and g). At 48 h after pruning, these buds showed considerable development

Fig. 3. Proton density and T2 images of buds 2, 5, and 12, 7 days after activation by TDZ treatment. (a) Proton density image of bud 2. (b) T2 image of bud 2. (c) Proton density image of bud 5. (d) T2 image of bud 5. (e) Proton density image of bud 12. (f) T2 image of bud 12. Note the loose bark, especially on bud 56 (e), in contrast to the much tighter bark on bud 12 (e). Explanation for colors and letters are the same as Fig. 1.

and the two buds developed almost equally (Fig. 1 b and d and f and h). The development was almost the same after 240 h (not illustrated), but, after that time, bud 5 did not develop any further, whereas bud 2 developed into a shoot (Table 1).

When the pruning cut was made below bud 4 of the medium-length shoots, bud 5 (now the top bud) (Fig. 2 a and c) had developed more slowly 48 h later than bud 2 of the long shoot (Fig. 1 b and d), and the retarded development of bud 8 (fourth bud below) was even more pronounced (Fig. 2 b and d).

As soon as the lateral buds were activated by removing the terminal bud, the water content of the stem and the bud increased (Fig. 1 b and f). Corresponding with the influx of water, metabolic activity increased, as evidenced by the enlargement of the bud: also, T2 values increased to the 10- to 20-ms range (Fig. 1 d and h). The stem at bud 5 retained more of the activated extra water (Fig. 1f), whereas bud 2 absorbed the water (Fig. 1b). The T2 values reflected the proton spin density images. In bud 2, higher T2 values were in the bud (Fig. 1d), whereas, in bud 5, the higher values (yellow and blue) were in the stem (Fig. 1h). The proton and T2 time picture did not change considerably from 2 days to 10 days after removing the terminal (not illustrated).

The development of bud 5 (now the top bud) of the medium-length stem after it was pruned was not only slower than that of bud 2 in a similar position on the long shoot, but the state of the water after 48 h did not change much (Fig. 2a vs. Fig. 1e). After pruning, T2 times in bud 5 on the short shoot (Fig. 2c) were relatively short, in the 15- to 20-ms range, with large areas showing purple and pink at the same location on respective images, indicating very short, or <5 ms, for T2 at these locations (Fig. 2c) (Millard et al., 1993).

Images of bud 8 of the medium-length shoot showed more water in the stem than those of bud 5 (Fig. 2b). This water did not move into the bud, which remained dormant, as evidenced by the short T2 times in the low-water areas (purple and pink areas together). Comparison of bud 5 on the long shoot and bud 8 on the medium shoot (the same bud position on shoots of different treatment) indicates a repeatable difference of more growth on the long shoot after pruning (Fig. 1 f and h vs. Fig. 2 b and d).

Buds were treated with TDZ without removing the terminal bud. For scheduling reasons, MRI images of such buds were taken 7 days after treatment. The treated buds developed rapidly and, after 7 days, buds 2 and 5 (Fig. 3 a and c) were larger than buds in similar position on long shoots induced by pruning 10 days after the terminal buds had been removed (not illustrated). As water moved into the buds, T2 values increased from the range of <10 ms to 20 to 30 ms. (Fig. 3 b, d, and f). Buds responded to TDZ treatment by being activated and producing shoots regardless of their position on the shoot (Table 1). Even though TDZ activated all buds, the growth of the lower buds was still slower, as illustrated by bud 12 (Fig. 3e). As lower buds developed more slowly than the upper buds, more water remained in the xylem of the stem and the bark and parts of the bud contained less water (Fig. 3 e vs. a).

In general, the less dormant the shoot, the easier the bark separates from the cambium layer. A separation of the bark due to handling the shoots and forcing them into NMR tubes is notable in some of the shoots supporting the upper buds and separation is very pronounced in shoots when the bud was treated with TDZ (Fig. 3 c and d).

Discussion

We have shown that, when paradormant buds resume growth, their free water content increases. However, paradormant buds have more free water than endodormant buds (Faust et al., 1991).

Regardless of how the buds are activated, either by removing the terminal bud or the upper part of the shoot or by application of TDZ, the response is essentially the same: There is more free water and it is freer in activated buds. T2 times increase in activated buds to the 30- to 40-ms range.

Also due to pruning, the initial swelling of the lower buds is not different from that of the upper buds (Fig. 1 b vs. f). Then, apparently, the upper buds take on the role of the removed terminal and prevent development of the lower buds into a shoot, but only after the initial burst of metabolic activity (Table 1). The reassertion of apical dominance appears to be between 2 and 10 days after removal of the terminal bud-before visible shoot growth occurs.

A gradient in dormancy along the shoot was observed. The ability of upper buds to grow appeared to be higher than that of lower buds. This phenomenon has been observed in other plants (Rubinstein and Nagao, 1976; Suzuki, 1990) and was explained by accumulation of inhibitory substances in the lower portion of the shoot as in the case of roses (*Rosa* sp.) (Zieslin and Halevy, 1976); by inhibition of actively growing shoot, as in the case of mulberry (*Morus alba* Linn.) (Suzuki, 1990); the presence of bud scales, as in black currant (*Ribes nigrum* Linn.) (Tinklin and Schwabe, 1970); or competition for nutrients and water (McIntyre, 1977). Our results indicate that the inhibitory gradient coincided with a gradient involving the total quantity and the quantity of bound water. Buds in the uppermost position along the shoot had a high water content and they contained proportionally more free water than did buds in the lower positions. Buds in lower positions contained proportionally more bound water than buds close to the terminal. The higher water content in the upper buds may reflect permeability changes in the stem-bud interphase because, in the lower buds, water apparently was available from the stem.

TDZ, a cytokinin analog, was able to overcome the inhibitory gradient and induce budbreak regardless of the position of the buds. However, a difference in growth response still existed. The higher buds (Fig. 3 a and b) developed faster than the lower buds (Fig. 3 e and f), even though TDZ was applied directly to the bud. This gradient in apple bud growth has been observed before (Wang et al., 1986). Therefore, it appears that, in addition to an inhibitory gradient, there exists also a growth potential gradient-both favoring the upper buds.

At this time, we do not know the function of this potential growth gradient. It may be related to more-advanced development of the upper buds at the time of activation. Nevertheless, activation and initial growth is reflected clearly in the state of water in the buds, and this phenomenon can be illustrated by MRI.

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