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Flower Bud Dormancy, ABA Concentration, and Survival during Frost of Jojoba Genotypes under Water Stress

Aliza Benzioni
Ben-Gurion University of the Negev, The Institutes for Applied Research, P. O. B. 1025, Beer-Sheva 84110, Israel

David A. Palzkill
Department of Plant Sciences, University of Arizona, Tucson, AZ 85721

John M. Nelson
Maricopa Agricultural Center, University of Arizona, 37860 West Smith-Enke Road, Maricopa, AZ 85239

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Abstract. **Flower bud dormancy and anthesis patterns, ABA concentration, and bud survival following frost were studied in eight jojoba *[Simmondsia shinensis* (Link) Schneider] clones grown under two irrigation regimes (water stress and well irrigated). Several clones broke dormancy in the autumn. Anthesis in the field before winter occurred only in one clone (a male) in the well-irrigated treatment. Buds on water-stressed plants broke dormancy earlier than those on well-watered plants, but anthesis in the field occurred later in the year. Buds on water-stressed plants were less affected by a severe frost than those on control plants (43% vs. 10% survival). There were large differences among clones in the amount of frost damage. ABA levels did not correlate with dormancy patterns or with the amount of frost damage. Chemical name used: S-(Z,E)-5-(1-hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl)-3-methyl-2,4-pentadienoic acid (ABA).**

Dormancy of jojoba flower buds is broken by exposure to temperatures between 5 and 20C (Dunstone, 1980). Clones differ in the duration of low temperature required to break dormancy. Flower buds released from dormancy will complete morphogenesis and proceed to anthesis only if water is available and plants have accumulated a sufficient heat sum (Benzioni and Dunstone, 1985; Ferriere et al., 1989). Clones vary in their chilling requirement, which may affect timing of anthesis. Environmental conditions known to affect the timing of anthesis include radiation level and availability of nutrients in the soil (Benzioni and Nerd; 1989; Dunstone 1988). Ferriere et al. (1989) and Milthorpe and Dunstone (1989) found that lines that broke dormancy late were more likely to survive frost episodes. Growers have observed that plants subjected to water stress in the autumn are more resistant to frost than those not stressed. Water stress may cause an increase in the chilling requirement by increasing abscisic acid, (ABA) levels in the buds (Ferriere et al., 1989). Thus, a high level of ABA may be correlated with resistance of flower buds to frost.

In this work we examined the effect of water Stress on the time at which flower buds broke dormancy, on flower bud ABA concentration, and on survival of flower buds after frost for eight jojoba clones.

Materials and Methods

Plant material. The experiment was carried out in 1989-90 in a jojoba plantation established from cuttings at the Univ. of Arizona, Maricopa Agricultural Center in 1984. The design was a split plot in randomized complete blocks with main plots replicated four times. The same female (1; 3; 4; 15; 21) and male clones (B; D; E) were represented in each of the main plots. Plant spacing was 1.2 × 4.1 m, with three rows of plants per irrigation block (main plot). The female clones had a relatively high yield in a field near Bakersfield, Calif. (Simons et al., 1989). The males had been selected from the same California field in such a way that pollen would be available throughout the whole female flowering period (early and late clones).

Irrigation regimes. Control plants were irrigated biweekly from 20 Mar. through 28 Nov. 1989. Irrigation was resumed on 20 Mar. 1990. The water supplied, calculated to replace soil water depletion as measured by a neutron probe, amounted to 518 mm in 1989. Water-stressed plants were flood irrigated twice (8 Mar. and 17 May 1989) with a total of 230 mm. A single irrigation was supplied on 20 Mar. 1990. Soil moisture content was measured weekly with a neutron probe at two locations in each irrigation plot. During the experimental period (Sept. 1989 to Feb. 1990), the soil moisture content of the control plots was much higher than that of plots that had been irrigated only twice in 1989 (Fig. 1).

Chilling requirement. Dormancy completion was estimated for the eight clones using the methods of Ferriere et al. (1989). Three plants per block, clone, and treatment were selected and tagged. A branch from each of the selected plants was harvested and placed in water in a growth chamber maintained at 24 ± 1C. Only buds that had already broken dormancy flowered in the growth chamber. Open flower buds were counted every 2 or 3 days for 24 to 27 days. Male buds were considered to be open when the anthers were visible, female buds, when the stigmas were visible. The number of days to 30% flowering in the chamber at 24C was calculated by use of linear regression and was then used to calculate the heat sum of growth degree heat (GDH). The number of GDH units in 1 day was calculated

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Abbreviations: BHT, butylated hydroxytoluence; GDH, growth degree heat.

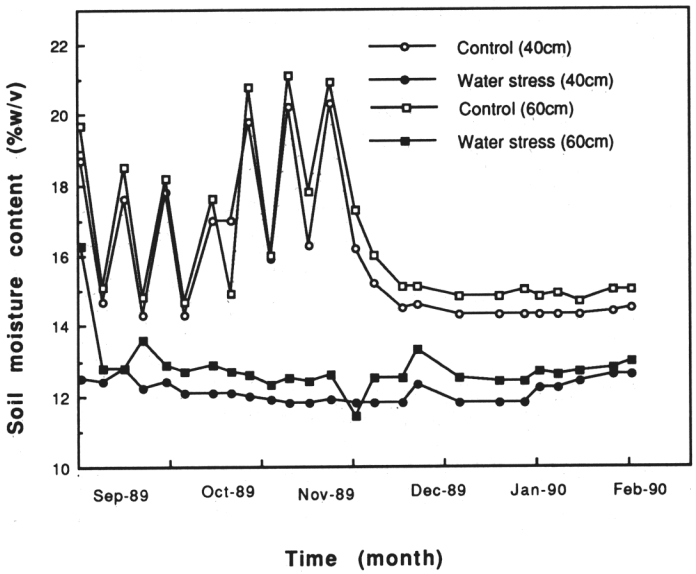

Fig. 1. Soil moisture content (percent weight to volume) at depths of 40 and 60 cm for each irrigation regime during the period of the experiment. A neutron moisture probe was used at two locations in each irrigation plot (n = 8).

as 264 GDH units [24 h × (24 to 13C), since 13C is the temperature at which jojoba ceases active growth].

ABA determination. On each harvest date (six times from September to February), flower buds from the same plants selected for estimation of chilling requirement were collected, immediately frozen on dry ice, and later lyophilized and stored at -80C. The buds were crushed in liquid N, and samples of 200 mg were ground in an extraction medium with an homogenizer and extracted overnight at 4C in 35 ml of 80% (v/v) methanol containing 200 mg of butylated hydroxytoluene (BHT) and 100 mg of ascorbic acid. ABA at 400 kBq of ^{14}C (+/-) was added to each extract to determine losses during the purification procedure. Methanol was evaporated off under a stream of N in a hood. The temperature of the extract during evaporation was kept at 30 to 35C by means of warm sand. The pH of the aqueous solution remaining after methanol evaporation was adjusted to 7.5 with NaOH, and the aqueous solution was extracted twice with 10 ml of hexane containing 300 mg BHT/liter. The aqueous phase was acidified to pH 2.8 with HCl and partitioned twice against 10 ml of dichloromethane containing 300 mg BHT/liter. The organic fractions were collected and further acidified with 1.5 ml of 1 mM HCl, and the dichloromethane was evaporated off. The aqueous solution remaining after dichloromethane evaporation was loaded onto a disposable, prepacked Waters-Sep-pak C18 cartridge (Waters, Milford, Mass.) preconditioned by aspirating 10 ml of methanol and 10 ml of 1 mM HCl in sequence. After loading, the cartridge was washed with 10 ml of water, and the ABA fraction was eluted with 1.6 ml of methanol. Fifty milligrams of BHT was added to the eluate, which was kept at -80C. Recovery was between 50% and 85%. ABA was quantified by ELISA using Phytodetek-ABA monoclonal antibody kits (Weiler, 1980).

Frost susceptibility. Temperature in the plantation was recorded at l-h intervals at several heights above ground throughout the experiment. After the frost of 16 Feb., buds that did not flower in the growth chamber within 27 days were dissected and their viability was assessed in terms of healthy ovary tissue. On 10 Mar., three branches per plant were selected in the field, and bud viability was re-evaluated. On 16 Apr., the number of open flowers, dormant buds, and dead flower buds were recorded in the field.

Results

Forced flowering in the growth chamber. One male clone (D) in the control treatment flowered (10% to 80% of the buds opened) in the field in October and November. The buds that did not open at that time remained dormant during the experiment and were sampled as were buds from the other clones.

Most of the buds that opened in the growth chamber did so within 12 to 14 days (Fig. 2). Although the branches in the chamber remained viable and even produced new leaves after that ‘time, most of the flower buds that had not flowered within 2 weeks remained dormant or they were desiccated. The percentage of buds of the clones that flowered within 24 days in the growth chamber on the various sampling dates differed significantly (Table 1).

Buds from various clones differed in the time at which dormancy was broken. We arbitrarily defined branches that had 30% flowering within 18 days (GDH of 4800) as having broken dormancy. By this definition, male clone E and female clone 15, which did not flower to any significant degree by February, did not break dormancy during the study period, while clone 4 and male clone D had already broken dorm; October in the water-stressed treatment and in November control treatment. Clones 1 and 21 had intermediate GDH values and clones 3 and B broke dormancy only in Jan. (Fig. 3). During December the readiness of buds to flower declined, perhaps due to the very low minimum temperatures and frosts that occurred during that month. A very severe : the night of 15 and 16 Feb. killed many buds, and the experiment was terminated.

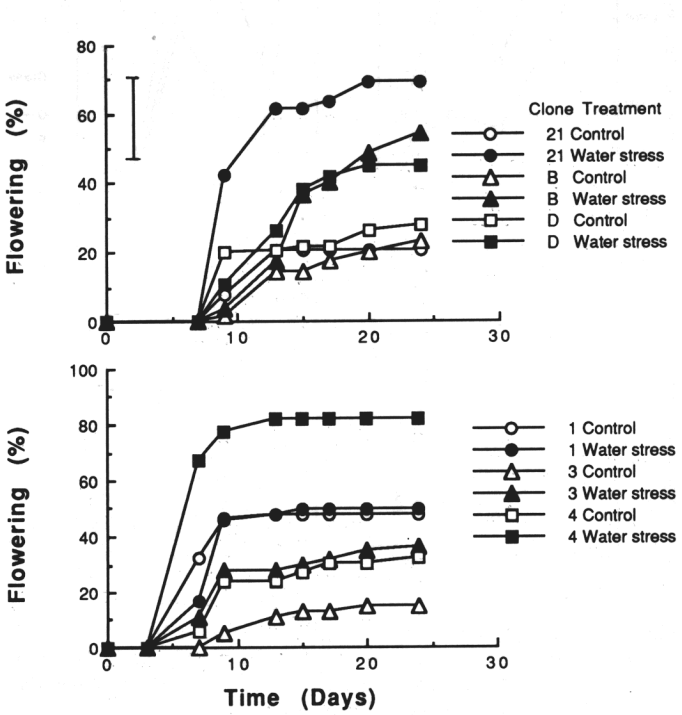

Fig. 2. Flowering pattern of detached branches of clones 21, B, D, 1, 3, and 4 harvested on 23 Jan. and forced in a growth chamber maintained at 24 ± 1C. Buds of the clones that are not represented in the figure did not flower on branches harvested by that date. Vertical bars represent the maximal critical range via Tukey.

Table 1. Percentage flowering of jojoba buds on detached branches kept for 24 days in a growth chamber at 24 ± 1C.

Clone	Water stress	Sampling date (1989–90) 29 Sept.	23 Oct.	21 Nov.	19 Dec.	23 Jan.	16 Feb.[z]
1	–	0	0	4.8 ± 4.1	3.9 ± 3.9	47.5 ± 14.5	Dead
1	+	8.8 ± 6.0	10.0 ± 6.0	0	4.3 ± 4.3	49.5 ± 13.9	86.1 ± 13.9
3	–	0	0	2.8 ± 2.8	0	14.7 ± 5.6	Dead
3	+	0	0	0	2.8 ± 2.8	34.6 ± 11.9	39.6 ± 20.4
4	–	0	4.2 ± 4.2	21.9 ± 7.5	14.9 ± 8.6	30.2 ± 13.3	Dead
4	+	31.7 ± 8.0	30.2 ± 12.2	15.2 ± 6.4	10.0 ± 6.3	82.0 ± 5.7	64.1 ± 23.1
15	–	0	0	0	0	0	Dead
15	+	0	0	0	0	0	Dead
21	–	0	0	1.8 ± 1.8	4.2 ± 2.7	20.5 ± 8.0	Dead
21	+	0	0	17.6 ± 11.0	2.7 ± 2.7	68.9 ± 11.5	97.5 ± 2.1
B	–	0	0	0	2.0 ± 2.0	20.0 ± 4.6	4.9 ± 2.5
B	+	0	1.7 ± 1.7	0	2.5 ± 2.5	54.2 ± 18.5	31.4 ± 10.9
D	–	1.9 ± 1.9	20.1 ± 4.9	55.0 ± 13.0	29.7 ± 9.3	26.6 ± 6.9	Dead
D	+	5.1 ± 5.1	27.2 ± 7.2	38.5 ± 8.6	33.6 ± 2.2	44.7 ± 6.4	85.0 ± 7.7
E	–	0	1.7 ± 1.7	6.0 ± 3.9	1.6 ± 1.6	1.8 ± 1.8	Dead
E	+	0	0	0	0	2.0 ± 2.0	2.4 ± 2.1
Significance							
Clone		NS	***	***	***	***	
Irrigation level		*	**	NS	***	***	
Interaction		NS	*	NS	NS	NS	
Critical range via Tukey		14.1	10.6	15.3	12.2	24.8	

[z]Freeze occurred in the field on the night of 15 and 16 Feb.
NS,*,**,***Nonsignificant or significant at $P < 0.05$, 0.01, or 0.001, respectively. Means of three branches from three plants in each of four blocks ±SE.

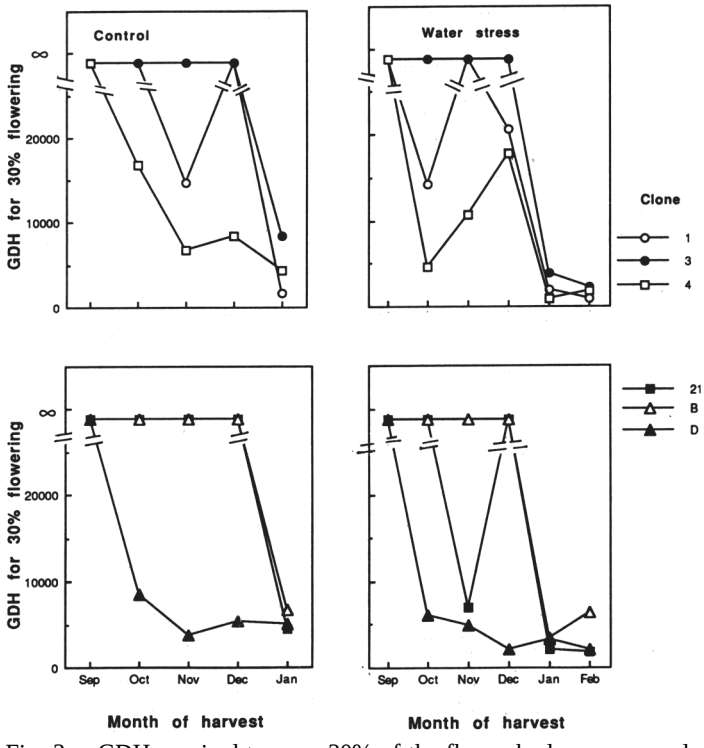

Fig. 3. GDH required to open 30% of the flower buds over several harvests for two male (B, D) and four female (1, 3, 4, 21) clones in the two irrigation treatments. One male (E) and one female (15) clone did not flower during the experiment.

Water stress enhanced flowering in the growth chamber for the six clones that flowered during the experiment. The water-stressed plants also flowered earlier than the well-irrigated ones and required a lower GDH for flowering (Table 1, Fig. 3).

ABA in flower buds. The average level of ABA in buds from water-stressed and control plants was similar throughout the experiment, with the highest ABA concentration being measured in September (1280 pmole/gdw) and the lowest in December (626 pmole/gdw). During January and February, ABA levels increased slightly over those in December. The eight clones differed in their ABA levels, clone B having consistently high levels and clone D having low ABA concentrations (Table 2). Chilling requirements and ABA levels were not correlated ($r^2 = 0.13$).

Survival of flower buds during frost. Irrigation regime had a clear effect on the survival of flower buds (Table 3). While 40% to 45% of the buds on the water-stressed plants survived, only 9% to 13% of the buds on the well-irrigated plants did so. Frost susceptibility of clones also differed significantly. In no case did water stress completely protect buds from frost damage, although some clones had better bud survival than others.

Discussion

The great variability in chilling requirements found among clones in this study is similar to that reported by Ferriere et al. (1989). The GDH values, although very different among the studied clones, were on average much higher than those reported for most of the Australian clones, possibly because the clones in Maricopa were selected for high yield; if lateness is associated with yield, some selection toward lateness had already been made. The only clone without a high GDH value was the male clone D, which was selected for its continuous flowering habit (Fig. 3, Table 1).

High chilling requirements may be the result of high levels of ABA in the dormant flower buds (Milthorpe and Dunstone, 1989). Although in this study the ABA levels in the various clones differed significantly, no simple relationship between ABA levels

Table 2. Concentration or ABA (pmole/gdw ± SE) in Jojoba flower buds of plants (combined from various clones) subjected to one of two irrigation regimes during the period of Sept. 1989 to Feb. 1990.

Water stress	Clone	Sampling date 29 Sept.	23 Oct.	21 Nov.	19 Dec.	23 Jan.	16 Feb.
−	4	1735 ± 160	991 ± 52	647 ± 53	513 ± 35	833 ± 55	ND
+	4	1456 ± 87	1479 ± 424	588 ± 66	593 ± 35	764 ± 234	ND
−	15	933 ± 75	822 ± 140	564 ± 34	646 ± 112	1130 ± 85	ND
+	15	1160 ± 194	973 ± 158	604 ± 44	530 ± 39	910 ± 74	ND
−	21	941 ± 79	490 ± 103	281 ± 41	720 ± 55	667 ± 53	ND
+	21	1120 ± 115	572 ± 59	288 ± 26	648 ± 39	809 ± 181	1332 ± 131
−	B	1868 ± 40	1213 ± 385	1282 ± 114	947 ± 187	737 ± 13	852 ± 72
+	B	1775 ± 368	1234 ± 133	1178 ± 100	799 ± 36	873 ± 179	979 ± 160
−	D	1194 ± 167	924 ± 68	991 ± 312	392 ± 21	526 ± 82	ND
+	D	999 ± 111	1018 ± 199	596 ± 33	522 ± 493	493 ± 25	ND
−	E	838 ± 197	1015 ± 87	857 ± 94	537 ± 94	699 ± 13	638 ± 87
+	E	1334 ± 182	1220 ± 22	752 ± 165	465 ± 37	569 ± 20	1108 ± 144

	Significance	Critical range via Tukey
Clones	***	95.5
Dates	***	145.5
Treatment	NS	
Inteactions	NS	

NS,*** Nonsignificant or significant at $P < 0.001$. ND = not determined.

Table 3. Percent viable flower buds in field near Maricopa, Ariz.

Clone	Water stress	Time of evaluation 15 Mar.[z]	16 Feb.[y]	16 Apr.[x]
1	−	0	1.3 ± 1.1	16.5 ± 6.8
1	+	43.0 ± 11.7	44.8 ± 16.0	60.2 ± 8.1
3	−	6.8 ± 6.8	4.0 ± 2.3	3.4 ± 1.9
3	+	42.6 ± 9.4	67.3 ± 15.6	51.6 ± 14.0
4	−	2.2 ± 1.4	8.7 ± 8.7	8.2 ± 5.6
4	+	38.2 ± 13.4	31.0 ± 14.3	43.9 ± 11.5
15	−	0	0	4.3 ± 3.6
15	+	40.2 ± 14.9	0	45.0 ± 14.8
21	−	0	2.0 ± 2.0	0.6 ± 0.1
21	+	23.3 ± 8.6	44.9 ± 20.8	64.7 ± 11.0
B	−	54.2 ± 8.9	58.9 ± 24.8	27.3 ± 8.9
B	+	63.3 ± 13.5	67.7 ± 18.7	60.1 ± 14.7
D	−	8.5 ± 3.3	0	0.9 ± 0.9
D	+	38.6 ± 12.8	29.7 ± 11.2	33.3 ± 9.3
E	−	40.0 ± 16.3	16.7 ± 15.7	24.3 ± 9.8
E	+	51.2 ± 13.2	52.9 ± 20.5	53.5 ± 14.4
Average	−	14.0 ± 7.4	11.5 ± 8.2	9.4 ± 2.9
	+	42.5 ± 4.0	42.3 ± 9.1	52.4 ± 4.9
Significance	Clone	(6.14)***	(6.45)***	(6.34)***
	Treatment	***	***	***
	Interaction	NS	NS	NS

[z]Evaluated in the field.
[y]Evaluated on branches collected on date and kept for 24 days at 24 ± 1c.
[x]Evaluated in the field.
NS,*** Nonsignificant or significant at $P < 0.001$, numbers in parentheses are critical range via Tukey.

and GDH values or lateness could be demonstrated (Table 2); for example, the early flowering clone 4 had relatively high ABA levels in September and October Thus, the control of dormancy and the effect of chilling require further study.

We hypothesized that water stress would postpone flowering by increasing the chilling requirement. The opposite was found: in the growth chamber, buds on branches from water-stressed plants required a lower GDH for 30% flowering and had a higher flowering percentage than those not stressed. It could be argued that upon release from water stress, the swelling of the flower buds proceeded at a faster rate, but then we would expect control plants to flower to the same extent, only slower. This was not the case (Fig. 2). Another possible explanation is that more of the buds on the water-stressed branches were viable. This situation is possible, at least for the later harvests from December on, after the three severe frosts that occurred during December.

As expected, water stress delayed flowering in the field. While all the well-irrigated plants from clone D flowered (between 10% to 80% of the buds were open) in the autumn, none of the water-stressed ones did so.

Water stress has been reported to inhibit or delay flowering (Benzioni and Dunstone, 1985; Benzioni and Nerd, 1989), and it has been suggested that it does so by increasing ABA levels in the buds (Benzioni and Dunstone, 1985; Ferriere et al., 1989). Since the evidence presented in these studies was circumstantial, we tried to examine ABA levels by a more direct approach. As had been found in Australia (Benzioni and Dunstone, 1985), ABA levels in the buds declined during early winter, but, unlike the continuous decline observed under the controlled environment used by Benzioni and Dunstone, the decline in our plantation was less dramatic. In our study, ABA levels stopped declining in early winter and then tended to increase. This change may have been the result of the extreme temperature fluctuations at Maricopa, where cold weather and frosty nights alternated with warm weather several times between December and February.

The effect of water stress on flowering in the field seems to be best explained in terms of its inhibition of the final steps of bud maturation and swelling. Thus, stressing plants in autumn may be effective only if no significant precipitation followed by warm weather relieves the stress before frost episodes. Thus, areas where heavy rains are expected and frosts are frequent, only clones with a high chilling requirement should be planted.

Ferriere et al. (1989) suggested that clones with high chilling

requirements are less susceptible to frosts. In the well-irrigated treatment, none of the female clones exhibited resistance to the severe frost of February, which killed 90% to 100% of the flower buds. The two late flowering males, B and E, had a survival rate of ≈ 40% to 50% even under well-watered conditions (Table 3).

Survival rates of buds on water-stressed plants were much higher than on control plants for all eight clones and can be ranked from the highest to the lowest as follows: 21 > 1 = B > E > 3 > 15 > 4 > D. Clones 4 and D had the lowest chilling requirements and were also the most susceptible to frost; they differed significantly from clones 1, B, and E in their viability. Although clones 3 and 15 were late in flowering, they were more susceptible to frost than other late-flowering clones, indicating that other mechanisms in addition to dormancy contribute to frost tolerance of jojoba flower buds.

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