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Possible Role of Volatile Fatty Acids and Abscisic Acid in the Dormancy of Oats

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ABSTRACT

Species of *Avena* differ markedly in their levels of pre- and post-harvest dormancy. These species offer the opportunity of determining if dormancy is related to the endogenous level of growth inhibitor. Germinability in two species of differing levels of dormancy, common oat *Avena sativa* L., and wild oat *Avena fatua* L. was assessed as were the contents of abscisic acid and volatile fatty acids of chain length C_6–C_{10}. In *A. sativa* which did not possess postharvest dormancy there was no correlation between germination and inhibitor levels but in *A. fatua* the relationship between the content of fatty acid and dormancy was good. The loss of these fatty acids in dry storage by evaporation could explain after ripening.

In oats (*Avena spp.*) there is a considerable range of degrees of dormancy from the almost nondormant common oat (*A. sativa* L.) which at most shows a brief postharvest dormancy, through the appreciable dormancy of the wild oat (*A. fatua* L.) to the profound dormancy of the winter wild oat (*A. ludoviciana* Dur.), especially with respect to the upper grain of the dissemule. The level of dormancy in this last species during maturation is less than when fully ripe (17, 21). However, Morgan and Berrie (17) showed that if immature grains were dried before testing these dried grains showed a degree of dormancy equivalent to the mature grain.

Wright (25) showed that water-stressed plants had elevated levels of an ABA-containing complex, possibly produced to control stomatal aperture (12). It might be concluded that ABA would accumulate in plant tissues in which the water contents were reduced compared to average normal tissues. Inasmuch as ABA is a well known germination inhibitor, such accumulation could explain the pattern of emergence found in maturing *Avena* spp.

With the possible exception of ABA, naturally occurring plant growth inhibitors are considered to be nonspecific (15). This includes the coumarins which can impose light sensitivity on lettuce seed (4).

Fatty acids of chain length C_6–C_{12} occur in many plants and influence seed germination and hydrolase production in a manner contrary to known promotive plant hormones (3, 9). We were interested in seeking a correlation between the content of fatty acid and physiological behavior in the germination of dormant and nondormant species of *Avena*. A similar correlation might exist between dormancy and ABA levels in oat grains and our objective was to test for such correlation.

MATERIALS AND METHODS

Both the cultivated and wild oats were grown at the experimental gardens of the University of Stirling. Batches for analysis were collected between June and August 1975 and these were assessed for dry weight, moisture content, and the endogenous levels of ABA and volatile fatty acids. A known weight of whole grains was taken at each collection. As the grains matured the proportion of kernel (caryopsis proper) to the rest (w/w) increased and the kernel would therefore contribute more to the values of these parameters as time passed. No attempt was made to separate the grain into its component parts. Dry weight was determined by holding grains at 60 C until constant weight was obtained and expressing this weight as a percentage of fresh weight.

Ripe grains were stored at a constant temperature of 20 C in darkness until required for analysis.

Single large batches of grain were collected. Prior to beginning the routine extractions, a model system employing replication at all levels was carried out for each acid. Isotope dilution was employed to estimate efficiency of extraction and recoveries were also estimated by "seeding" samples with known amounts of acid. From these experiments the coefficient of variation for each acid was determined. In the working analyses the final analysis was carried out in triplicate and the average of these was employed, using the appropriate coefficient of variation to estimate the standard deviation. It is this value which is reported in the tables.

Extraction and Analysis of ABA. ABA was extracted by the method of Berrie *et al.* (3). The levels of inhibitor in the extracts were determined as MeABA[4] by GC employing an electron-capture detector. A Perkin-Elmer F17 gas chromatograph using the following stationary phase and settings was used:

Stationary phase	:	1.5% FS-1265 (QFI) coated on 60–80 Chromosorb W AW-DCMS
Column	:	1.5 m × 3 mm (stainless steel)
Oven temperature	:	Isothermal 210 C
Injector detector temperature	:	275 C
Carrier gas	:	N_2 at 60 ml min^{-1}

Extraction and Analysis of Volatile Fatty Acids. Ground grains were extracted by the Soxhlet procedure employing a preextraction using 40 to 60 C bp petroleum ether to remove nonpolar lipids. The acids proper were extracted using methanol. Redistilled solvents were used in these extractions. On completion of the extraction a small volume of water was added and the methanol removed by rotary evaporation at 30 C. The aqueous phase that

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[4] Abbreviation: MeABA: methyl abscisate.

remained was acidified with 10 M H_2SO_4 to pH 2.0 and partitioned against diethyl ether (three partitionings).

In the case of the common oat the aqueous solution obtained after methanolic extraction and rotary evaporation was steam-distilled against ether vapor and the volatiles collected in the ether phase.

Steam distillation of the aqueous solution obtained from the common oat was adopted because of problems associated with subsequent partitioning against acid. While introducing an additional stage it gave results similar to the standard procedure both with regards to the content of fatty acid and efficiency of extraction as determined by isotope dilution using ^{14}C-labeled nonanoic acid. Neither method was found to be very efficient with an over-all effiency of about 25%. All values reported in this paper, for the amounts of acid present in the grains have been corrected to take account of the extraction procedure used. The efficiency of extraction of ABA, as determined by isotope dilution, ranged from 60 to 70% and again the values reported were corrected to take account of extraction and analysis efficiencies.

These ethereal solutions were then partitioned against 5% $NaHCO_3$ (pH 8.8) three times. The aqueous phases which were obtained were bulked and adjusted to pH 2.0 with 10 M H_2SO_4 and the free acids removed by partitioning against ether. After dehydration this ethereal solution was reduced to a small volume and applied to a column containing 15 g of silica gel (100–200 mesh) treated with 30 ml of alkaline isopropyl alcohol (~50 mg KOH ml^{-1}) and the acids eluted after the method of McCarthy and Duthie (13). Neutral materials were removed from the column (8 × 1.5 cm) with 100 ml ether and were discarded. The acids were next eluted with 50 ml of 2% formic acid in ether and a further 100 ml of ether. This total of 150 ml contained the acids. The solvent was removed and the acids methylated with diazomethane prior to GC (2).

Routine analyses of the levels of fatty acids in extracts were carried out by GC using a Perkin-Elmer F17 gas chromatograph set up as follows:

Stationary phase	:	5% FFAP coated on 80-100 Chromosorb G AW-DCMS.
Column	:	1.8 m × 3 mm stainless steel
Oven temperature	:	Isothermal 135 C
Injector/detector temperature	:	175 C
Carrier gas	:	N_2 at 35 ml min^{-1}
Air/H_2 pressures	:	165 and 100 KNm^{-2}

The acids were unequivocally identified by MS using either an AEI MS-30 or JOEL D100 mass spectrometer combined with a gas chromatograph.

Characterization was also supported by coinjection of known standards.

Germination Tests. Fifty grains were spread over the surface of a moistened (7 ml) 9-cm Whatman No. 3 filter paper lining the base of a 9-cm Petri dish. The dishes were incubated at 20 C ± 1 C in darkness, being wrapped in foil. Germination was assessed as radicle emergence at 72 h and 168 h. Three replicates were employed.

RESULTS

Changes in fresh weight, dry weight, and moisture content in *A. sativa* and *A. fatua* during grain maturation are shown in Figures 1, A and B, respectively. The expected increase in dry weight and the decline in moisture content were as expected for both species but the pattern of grain filling differed. In *A. sativa* grain filling proceeded almost uniformly from anthesis until maturity at 63 days, a daily increase of about 0.5 mg, but in *A. fatua* (Fig. 1B) noticeably grain filling did not begin until about 30 days after anthesis. From 30 to about 60 days the grain dry weight increased from about 2 mg to 16.8, an increase of ~0.5 mg/day.

As each batch was collected to determine the course of grain development samples were assayed for germinability. In the case of *A. sativa* the data showed a trend of increasing germination. It can be seen that the capacity to germinate increased to a given level during maturation on the plant and that there was a second phase of development of germinability associated with storage and that this could be considered the after-ripening stage. Wild oats did not show a propensity to germinate until they had been stored for at least 5 months after harvest (Fig. 2).

It might be supposed that the course of change in capacity to germinate would be regular and than any internal factor correlating with germination would likewise show regular changes. Because of the sampling procedure, fluctuations will be present but any regularity can be made more apparent by employing running means. This technique has been avoided in presenting the data but often in the argument the trends discussed can be more readily envisaged by employing this method of data presentation.

It was considered better to represent ABA levels on a per grain basis since this might be more important biologically than the

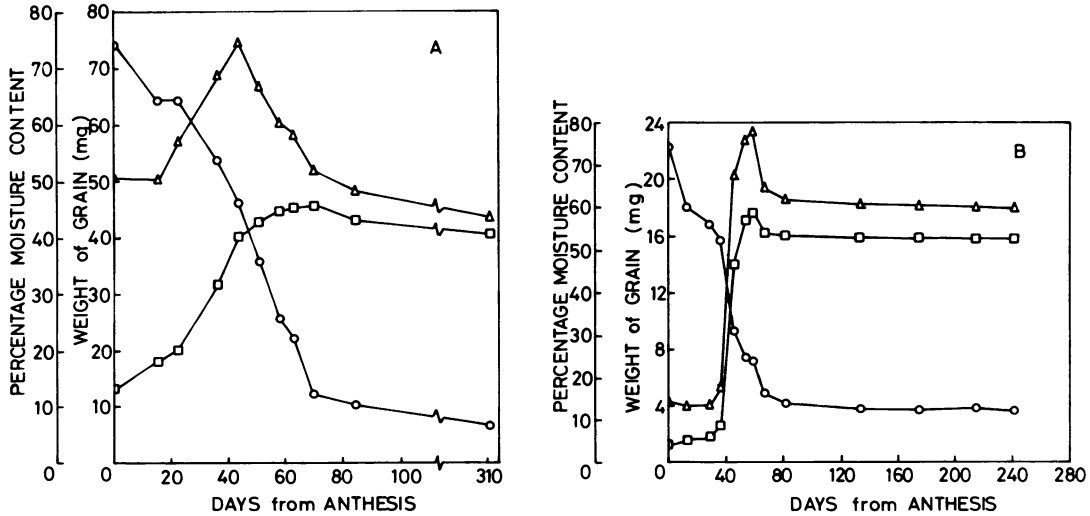

FIG. 1. A: course of change of weight and moisture content in *A. sativa* from anthesis. Harvest was at 63 days from anthesis. B: course of change of weight and moisture content in *A. fatua* from anthesis. Harvest was at 67 days from anthesis. (△——△): Fresh weight; (□——□): dry weight; (○—○): moisture content (three replicates).

amounts of ABA per unit weight. In Table I we see the changes in free, neutral bound, and acid-bound ABA which were measured in *A. sativa* during maturation. Initially, very high levels of free and acid-bound ABA were observed, during the period in which there was maximum increase in dry weight, but these forms declined as the grain matured. At harvest the level of neutral bound ABA increased substantially to decline during the immediate postharvest period.

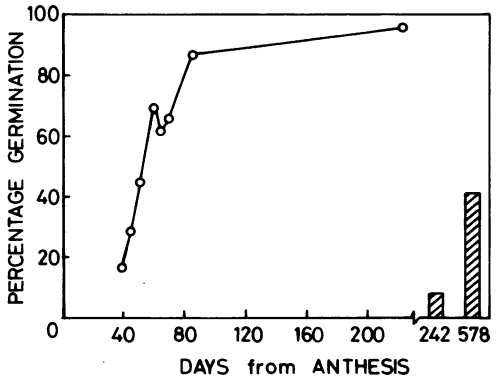

FIG. 2. Development of capacity to germinate in *A. sativa* (graphs) and *A. fatua* (histogram) from anthesis.

It is difficult to discern any trends in the levels of ABA (total, free, acid-bound, or neutral bound) during maturation or immediately postharvest. Although ABA was not detected at harvest it is unlikely that it was absent and similarly at the last analysis undertaken. Very high levels of ABA were encountered during the first phase after anthesis. During the period of grain fill the amount of ABA varied by a factor of ~3 on a per grain basis but not exhibiting any regularity. On a weight basis ABA levels tended to decline. If the ABA is localized, then expressing content on a per grain basis is meaningful but if it is distributed generally throughout the grain the content is best expressed on a unit weight basis.

The inability of common oat to germinate 19 days after anthesis may have been due to the high level of ABA (both on a weight and per grain basis) but could also be explained on the very small degree of embryo development which had occurred by that time.

The moisture content of the grain at harvest (~17%) was sufficiently high to permit metabolism to take place and the changes in ABA levels at 70 and 77 days could be due to metabolic events associated with storage conditions in the immediate postharvest period.

The pattern of ABA levels in wild oats is clearer. All three

TABLE I Percentage germination and levels of ABA in grain of Avena sativa during maturation and subsequent dry storage. (Values given are corrected for extraction losses and are the calculated amounts for individual observations for the dry seed.)

		FREE ABA			"NEUTRAL" BOUND			"ACID" BOUND			TOTAL	
Days from anthesis	% age Germination	μg kg^{-1}	pg grain^{-1}	% total ABA	μg kg^{-1}	pg grain^{-1}	% total ABA	μg kg^{-1}	pg grain^{-1}	% total ABA	μg kg^{-1}	pg grain^{-1}
19	0	52.3	1005	25	16.4	315	08	141.2	2714	67	209.9	4034
27	0	7.3	170	25	5.3	123	18	17.2	399	57	29.8	692
37	18.7	15.0	478	28	16.7	532	31	22.2	708	41	53.9	1718
44	29.3	3.3	132	26	7.9	317	61	1.7	68	13	12.9	517
51	45.0	8.8	376	88	1.23	53	12	UD	-	-	10.0	429
58	69.3	14.9	666	46	11.03	493	34	6.7	299	20	32.6	1458
63+	61.3	UD	-	-	UD	-	-	UD	-	-	UD	-
70	65.3	5.1	232	09	37.5	1709	70	11.3	515	21	53.9	2457
77	73.3	UD	-	-	UD	-	-	UD	-	-	UD	-

\+ Harvest date
UD Undetected

TABLE II Percentage germination and levels of ABA in grain of Avena fatua during maturation and subsequent dry storage. (Values given are corrected for extraction losses and are the calculated amounts for individual observations for the dry seed.)

		FREE ABA			"NEUTRAL" BOUND			"ACID" BOUND			TOTAL	
Days from anthesis	% age Germination	μg kg^{-1}	pg grain^{-1}	% total ABA	μg kg^{-1}	pg grain^{-1}	% total ABA	μg kg^{-1}	pg grain^{-1}	% total ABA	μg kg^{-1}	pg grain^{-1}
14	0	35.9	57	25	25.0	40	18	81.9	131	57	142.8	228
29	0	123.6	221	62	29.3	52	15	46.8	84	23	199.7	357
36	0	8.8	23	69	1.6	4	12	2.4	6	19	12.8	33
46	0	78.8	1109	50	29.4	414	19	48.3	680	31	156.5	2203
52	0	15.9	271	53	4.8	82	16	9.0	154	30	29.7	507
59	0	25.4	450	73	2.3	41	07	7.1	126	20	34.8	617
67+	0	7.5	121	66	2.8	45	25	1.0	16	09	11.3	182
81	0	19.9	319	80	2.8	45	11	2.1	34	09	24.8	398
133	0	15.4	246	81	1.7	27	09	2.0	32	10	19.1	305
175	0	9.8	156	84	.4	6	03	1.5	24	13	11.7	186
214	0	6.5	103	79	.4	6	05	1.3	21	16	8.2	130

\+ Harvest date

TABLE III Levels of hexanoic acid in oats at different stages of maturity and during post harvest storage at 20 C in darkness.

Avena sativa				Avena fatua			
Hexanoic acid				Hexanoic acid			
Days from anthesis	mg kg^{-1}	ng $grain^{-1}$	% of total VFA C_6-C_{10}	Days from anthesis	mg kg^{-1}	ng $grain^{-1}$	% of total VFA C_6-C_{10}
16	2.46 ± 0.14	57	18	14	0.38 ± 0.02	1	3
23	7.86 ± 0.44	205	20	29	2.49 ± 0.14	12	4
37	0.94 ± 0.05	39	13	36	1.71 ± 0.16	5	4
44	0.21 ± 0.01	11	2	46	2.99 ± 0.17	42	2
51	0.17 ± 0.05	7	1	52	10.63 ± 0.62	181	9
58	1.52 ± 0.09	87	6	59	14.40 ± 0.83	203	4
63+	1.88 ± 0.05	107	62	67+	6.89 ± 0.40	111	2
70	1.31 ± 0.04	75	62	81	8.63 ± 0.50	138	3
77	2.35 ± 0.07	132	64	133	9.04 ± 0.52	144	5
105	1.79 ± 0.05	97	70	175	4.25 ± 0.15	68	3
119	3.01 ± 0.09	163	67	214	2.54 ± 0.15	40	3
135	0.77 ± 0.02	41	59	242	0.89 ± 0.05	14	3
148	1.27 ± 0.04	68	49	578	0.44 ± 0.03	7	6

+ Harvest date

TABLE IV Levels of heptanoic acid in oats at different stages of maturity and during post harvest storage at 20 C in darkness.

Avena sativa				Avena fatua			
Heptanoic acid				Heptanoic acid			
Days from anthesis	mg kg^{-1}	ng $grain^{-1}$	% of total VFA C_6-C_{10}	Days from anthesis	mg kg^{-1}	ng $grain^{-1}$	% of total VFA C_6-C_{10}
16	0.57 ± 0.03	13	4	14	0.17 ± 0.01	trace	1
23	1.60 ± 0.05	41	4	29	0.16 ± 0.01	trace	2
37	0.39 ± 0.02	16	5	36	1.12 ± 0.08	3	2
44	0.05 ± 0.01	2	-	46	1.31 ± 0.16	18	1
51	2.26 ± 0.07	124	18	52	5.52 ± 0.41	94	5
58	0.07 ± 0.01	4	-	59	24.79 ± 1.84	439	8
63+	0.10 ± 0.01	6	3	67+	48.12 ± 3.58	778	17
70	0.13 ± 0.02	8	6	81	38.12 ± 2.12	610	15
77	0.56 ± 0.03	32	16	133	19.10 ± 1.42	306	12
105	0.19 ± 0.02	11	8	175	6.43 ± 0.48	102	5
119	0.56 ± 0.03	32	13	214	2.09 ± 0.15	33	3
135	0.19 ± 0.02	11	16	242	0.21 ± 0.02	3	1
148	0.56 ± 0.03	32	22	578	0.16 ± 0.01	7	6

+ Harvest date

forms rose as the grain began to fill. A maximum was reached prior to maturity, at 46 days, followed by a decline but in this case there was a persistence of ABA in storage especially free ABA (Table II). Wild oat germination cannot be seen to be as clearly dependent on the level of this inhibitor since the first evidence of germination at 242 days was associated with an amount of free ABA akin to that found at harvest, 67 days from anthesis.

When the levels of fatty acids C_6–C_{10} are considered in *A. sativa* and *A. fatua*, a very different pattern is seen, as in Tables III through VII. Because of the low individual grain weight of *A. fatua* early in grain fill (29 days) the grain content of fatty acids was low, at about 27 ng, whereas in *A. sativa* with its larger grain at about this time (23 days) it was 1020 ng. However, as the wild oat grains matured and enlarged the level in them increased dramatically until harvest when we found 4.482 μg of fatty acid in contrast to a level of 172 ng in common oats. At this time the fatty acid content of wild oat on a weight basis was even more pronounced than in common oat.

The changes in relative levels of the individual acids differed markedly between the two species. In common oat decanoic acid was initially the most prominent, but the postharvest phase hexanoic became the major acid present. In the immediate postharvest period octanoic was not present in large amounts. Heptanoic and nonanoic acids showed the same pattern, declining to harvest then increasing in the postharvest period.

Within the wild oats the acids showed quite different courses of relative change. Decanoic, which was the major acid at all times, declined slightly to harvest, then rose in the postharvest phase to the initial levels. Hexanoic, heptanoic, and octanoic were minor components with only heptanoic showing a marked maximum at

TABLE V Levels of octanoic acid in oats at different stages of maturity and during post harvest storage at 20 C in darkness.

Avena sativa Octanoic acid				*Avena fatua* Octanoic acid			
Days from anthesis	mg kg^{-1}	ng $grain^{-1}$	% of total VFA C_6-C_{10}	Days from anthesis	mg kg^{-1}	ng $grain^{-1}$	% of total VFA C_6-C_{10}
16	UD	-	-	14	0.76 ± 0.06	1	3
23	1.69 ± 0.08	44	4	29	0.36 ± 0.03	1	9
37	UD	-	-	36	0.25 ± 0.02	1	1
44	0.69 ± 0.03	35	7	46	2.19 ± 0.18	31	2
51	0.18 ± 0.01	10	1	52	11.37 ± 0.95	194	9
58	0.49 ± 0.02	28	2	59	17.46 ± 1.46	310	6
63+	0.34 ± 0.01	20	12	67+	13.45 ± 1.13	218	5
70	0.33 ± 0.01	20	16	81	17.27 ± 1.44	277	7
77	0.47 ± 0.02	27	13	133	13.86 ± 1.16	221	8
105	0.21 ± 0.01	12	8	175	7.66 ± 0.64	122	6
119	UD	-	-	214	3.65 ± 0.31	58	5
135	0.04 ± 0.01	2	3	242	1.87 ± 0.16	30	7
148	0.55 ± 0.02	30	22	578	0.57 ± 0.06	9	8

\+ Harvest date
UD undetected

TABLE VI Levels of nonanoic acid in oats at different stages of maturity and during post harvest storage at 20 C in darkness.

Avena sativa Nonanoic acid				*Avena fatua* Nonanoic acid			
Days from anthesis	mg kg^{-1}	ng $grain^{-1}$	% of total VFA C_6-C_{10}	Days from anthesis	mg kg^{-1}	ng $grain^{-1}$	% of total VFA C_6-C_{10}
16	2.27 ± 0.10	52	17	14	0.95 ± 0.08	1	7
23	14.97 ± 0.67	383	37	29	0.85 ± 0.07	2	11
37	0.52 ± 0.02	21	7	36	8.20 ± 0.69	22	17
44	1.45 ± 0.06	73	14	46	23.35 ± 1.96	329	17
51	0.96 ± 0.04	52	8	52	41.65 ± 3.5	710	36
58	UD	-	-	59	99.10 ± 8.32	1758	33
63+	0.10 ± 0.01	5	3	67+	90.35 ± 7.58	1462	33
70	0.31 ± 0.02	18	15	81	60.65 ± 5.09	971	24
77	0.24 ± 0.01	14	7	133	30.70 ± 2.58	490	19
105	0.34 ± 0.02	19	14	175	32.45 ± 2.72	516	25
119	0.84 ± 0.04	47	19	214	9.95 ± 0.84	143	12
135	0.28 ± 0.01	16	22	242	1.00 ± 0.08	16	4
148	0.19 ± 0.01	11	8	578	0.60 ± 0.05	9	8

\+ Harvest date
UD undetected

harvest although octanoic reached a high harvest value which was maintained on storage. Nonanoic exhibited a relative pattern of content like that of heptanoic.

When absolute levels of acid are examined, the acids each showed the rise to harvest but to differing degrees: C_6, 12.2 × s; C_7, 77 × s; C_8, 36.5 × s; C_9, 25 × s; C_{10}, 7.4 × s.

DISCUSSION

It has been suggested that dormancy in *A. fatua* is controlled by inhibitors (6, 7, 18) and that release from dormancy is occasioned by an interaction between these inhibitors and promoters (19, 21). Andrews (1) proposed that ABA may be present in dormant embryos of wild oat and the volatile acids have been detected in grains of both cultivated and wild oat (3). Either, both, or neither of these inhibitors could be involved in the control of dormancy and germination in *Avena* spp.

McWha (14) and King (10) have shown ABA to be present in developing wheat and King suggested that the dramatic rise (40-fold) in the content of this inhibitor in the period prior to rapid desiccation prevented precocious sprouting. In wheat both of these workers found the level of ABA at its peak to be about 6 to 8 ng per grain. This is substantially more than we have found in either of the oats yet *A. fatua* is less liable to precocious sprouting and has a deeper dormancy than wheat, and the common oat is little different in its behavior compared to wheat.

In other species which exhibit dormancy it is difficult to obtain a correlation between the endogenous level of ABA and the physiological state of the seed. Braun and Khan (8) and Berrie and Robertson (5) were unable to associate endogenous levels of

TABLE VII Level of decanoic acid in oats at different stages of maturity and during post harvest storage at 20 C in darkness.

Avena sativa				Avena fatua			
	Decanoic acid				Decanoic acid		
Days from anthesis	mg kg^{-1}	ng grain^{-1}	% of total VFA C_6-C_{10}	Days from anthesis	mg kg^{-1}	ng grain^{-1}	% of total VFA C_6-C_{10}
16	11.18 ± 2.42	186	61	14	6.61 ± 0.48	15	86
23	19.79 ± 4.28	372	36	29	21.04 ± 1.52	12	74
37	7.70 ± 1.66	227	75	36	37.22 ± 2.69	98	77
44	11.09 ± 2.40	411	77	46	107.48 ± 7.76	1510	78
51	12.18 ± 2.64	482	71	52	46.60 ± 3.37	794	40
58	34.26 ± 7.41	1416	92	59	151.65 ± 10.95	2684	50
63+	0.58 ± 0.02	34	20	67+	118.43 ± 8.56	1913	43
70	UD	-	-	81	125.74 ± 9.08	2010	50
77	UD	-	-	133	93.04 ± 6.72	1481	56
105	UD	-	-	175	78.61 ± 5.68	1248	61
119	UD	-	-	214	57.74 ± 4.17	914	77
135	UD	-	-	242	24.17 ± 1.74	382	86
148	1.06 ± 0.04	-	-	578	9.04 ± 0.65	83	73

+ Harvest date
UD undetected

ABA and germinability in lettuce. In *Fraxinus americana* embryo growth occurred when ABA content was high (24). Sondheimer *et al.* (23) could not correlate ABA content and the germination of this cold-requiring species.

Changes in the proportions of free and bound ABA may be more important in regulating growth processes although Milborrow and Noddle (16) suggested that any changes in these proportions that might be capable of controlling growth processes are due to synthesis of ABA rather than interconversion.

Bound ABA is probably a water-soluble glucose derivative possessing half the activity of free ABA (11) and it is probable that the production of this and other esters is a "detoxification" mechanism (20). Among the bound fractions we obtained there are likely to be artifactual compounds arising during extraction and work-up by processes such as transesterification (15).

Whereas it is not possible to correlate ABA content and physiological state the same cannot be said for the volatile fatty acids. The common oat, essentially nondormant, has relatively low levels of fatty acid but the wild oat has much elevated levels particularly at harvest.

From our previous work (3) the acids shown to be most effective in reducing germination are C_7, C_8, and C_9, the others C_6, C_{10} being much less so. At 37 days from anthesis when the common oat showed ~19% germination the content of acids was 303 ng per grain. Germination continued to rise as the grain matured, as did the acid level, but this increase was due almost wholly to C_{10} an acid shown to be ineffective in inhibiting germination. The most effective inhibitor, C_9, decreased to insignificant levels. At harvest and during postharvest storage, germination was high and the levels of acids were low.

The situation in wild oat was different. Here there was a gradual build-up of acids during maturation and when the grain-filling period was considered, *A. fatua* had substantially more acid present, especially C_9, at a time when it could be assumed that the grain was morphologically sufficiently developed for an embryo to grow out subsequent to germination. The low levels present immediately up until 36 days after anthesis had no influence on germinability because the embryo was too small.

After-ripening may be due to a loss of inhibitor during grain storage. The dry grain was not known to exhibit marked metabolic activity and although there was a reduction in the amount of ABA in wild oats stored 105 days after harvest large changes in ABA levels seemed only to take place when the grain was hydrated.

With the volatile fatty acids the loss in storage was large, declining from 2,458 to 25 ng per grain for the C_7, C_8 and C_9 acids. The loss of these acids could be brought about by metabolism, chemical interconversion or degradation, or evaporation. The desiccated condition of the grain in storage probably excludes the first mechanism as a major contributor to the loss. Chemical degradation cannot be excluded but we favor a simple evaporative loss. Certainly the level of C_9 stored seed followed a simple decay pattern with a half-life of 56.5 days. Heptanoic and decanoic acids decreased in amount in stored seeds in a similar fashion but not exhibiting a simple decay. The fact that after-ripening is accelerated at high temperatures supported the evaporation theory.

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