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Role of Endogenous Growth Regulators in Seed Dormancy of *Avena fatua*

I. SHORT CHAIN FATTY ACIDS

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JAMES D. METZGER[1] AND DEBORAH K. SEBESTA
Metabolism and Radiation Research Laboratory, United States Department of Agriculture, Agricultural Research Service, State University Station, Fargo, North Dakota 58105

ABSTRACT

The hypothesis that endogenous short chain fatty acids (C 6–C 10) are important in maintaining seeds of wild oat (*Avena fatua* L.) in the dormant state by acting as natural germination inhibitors (Berrie, Buller, Don, Parker, 1979 Plant Physiol 63: 758–764) was investigated. When germination of nondormant seeds was inhibited by treatment with short chain fatty acids, the seeds did not revert to a similar biochemical and physiological state as exhibited by dormant seeds. First, nonanoic acid-induced inhibition of seed germination was not reversed by hormone treatments which normally break dormancy in wild oat seeds. Second, nondormant seeds treated with short chain fatty acids maintained similar relative proportions of the pentose phosphate pathway and the Embden-Meyerhoff-Parnas pathway for respiratory glucose metabolism as that found in the nondormant controls. Seeds imbibed in the presence of nonanoic acid lost more amino acids and proteins into the imbibition solution than did the untreated controls, suggesting membrane damage had occurred. Inasmuch as increasing concentrations of nonanoic acid also progressively reduced the growth of the coleoptile and roots of intact seedlings until all growth ceased and no germination occurred, the inhibition of seed germination could be due to a nonspecific inhibition of growth of the embryo, perhaps because of disruption of membrane structure and function. Finally, no correlation between endogenous levels of short chain fatty acids in seeds or isolated embryonic axes and seed dormancy could be demonstrated.

Seeds of wild oat (*Avena fatua* L.) are normally dormant at the time of dehiscence from the parent plant; that is, even though fully viable, dormant seeds will not resume growth when supplied conditions that support germination of nondormant seeds (21). Upon dry storage, dormant seeds undergo physiological and biochemical changes, termed afterripening, which cause the transition from a dormant to a nondormant state.

Endogenous growth regulators have been implicated in the induction, maintenance, and termination of dormancy in seeds of *A. fatua* (21). Recently, it has been proposed that volatile fatty acids of chain length C 6–C 10 play a major role in the induction and maintenance of seed dormancy in wild oat by acting as natural germination inhibitors (2). The reasoning behind this hypothesis was 3-fold: first, fatty acids, particularly C 7, C 8, and C 9, were moderately effective inhibitors of seed germination in *A. fatua* (3). Second, the endogenous levels of fatty acids in dormant seeds were in the range necessary for inhibition of germination of nondormant seeds. Third, and most importantly, there was a strong correlation between the levels of endogenous short chain fatty acids and the degree of dormancy in seeds of wild oat (2). These workers suggested that loss of the fatty acids by evaporation from the seeds could explain the basic mechanism underlying the afterripening process (2).

Implicit in this work is the assumption that nondormant seeds, inhibited from germination by exogenous short chain fatty acid, had reverted to a similar biochemical and physiological state as dormant seeds. Therefore, the inhibition of germination by an exogenous compound cannot be due to nonselective destruction of seed viability if the compound is to be considered a natural dormancy-inducing agent. With this criterion in mind, we compared the physiological and biochemical states of dormant seeds and nondormant seeds in which germination was inhibited from germination by short chain fatty acids. We also tested the hypothesis that evaporation of short chain fatty acids from seeds was responsible for the termination of dormancy.

MATERIALS AND METHODS

Seed. Wild oat seeds (*Avena fatua* L.) were collected locally in North Dakota and afterripened 2 years. These seeds were completely nondormant and had a germination rate >95%. Plants from this seed grown to maturity in the greenhouse produced dormant seeds (initial germination, <5%; application of 10^{-4} M gibberellin A_3 increased germination to >95%). In experiments involving dormant seeds, freshly harvested seed (<1 month after harvest) were used.

Germination Tests. Five replicates of 20 seeds each were dehulled and placed embryo-side-down in a Petri dish lined with a piece of blotter paper that was soaked with 5 ml of a test solution. The germination tests were conducted in the dark at 25°C. After 4 days, germination was assessed. The seeds were considered germinated when the coleorhiza protruded through the testa.

Emulsions of NA[2] were prepared in aqueous 0.1% (v/v) Tween 20. The mixtures were sonicated for 30 min at room temperature. The resulting emulsion was stable for at least 1 week. The pH of the test solutions was adjusted to 4.8 with 6 N KOH before the start of the imbibition. The NA was redistilled under reduced pressure prior to use. Tween 20 alone did not have a detectable effect in any of the various biological tests performed in this study.

Glucose Utilization. The relative proportion of glucose utilization by seeds in either the EMP pathway or the PP pathway was determined by the method of Simmonds and Simpson (20). One hundred excised wild oat embryos were placed in a 50-ml Warburg

[1] To whom correspondence should be addressed.

[2] Abbreviations: NA, nonanoic acid; PP, pentose phosphate; EMP, Embden-Meyerhoff-Parnas.

flask with 1.5 ml of a solution containing 10^6 dpm of either [1-^{14}C]- or [6-^{14}C]glucose (specific radioactivity = 60 mCi mmol^{-1}, Amersham) and 0.1% (v/v) Tween 20 at pH 4.8. The flasks were outfitted with a center well which contained 0.4 ml 20% (w/v) KOH in H_2O. The flasks were sealed and solution was transferred to a scintillation vial containing 10 ml Instagel scintillation cocktail (Packard), and the amount of radioactivity as $^{14}CO_2$ absorbed by the KOH solution was determined by liquid scintillation spectrometry.

Measurement of Membrane Integrity. The loss of amino acids and proteins from seeds into the imbibition solution was used as a measure of membrane integrity (16). Fifty dehulled seeds were placed in a 50-ml flask containing 2.5 ml of a test solution at pH 4.8. The flasks were stoppered and incubated in the dark at 25°C. After 14 h, the imbibition solution was removed and clarified by centrifugation, and the ninhydrin-positive substances were quantified by the method of Rosen (17).

Coleoptile Straight Growth Test. The effects of fatty acids on auxin-induced growth in excised wild oat coleoptiles were assessed by the method of Shimabukuro *et al.* (19) except that all test solutions contained 0.1% (v/v) Tween 20 and 1 μM IAA. Each treatment was performed in triplicate.

Kinetics of Coleoptile Growth. Auxin-induced coleoptile growth of cultivated oat (*Avena sativa* L.) was measured continuously with a linear variable displacement transducer. The apparatus was a modification of those described by Green and Cummins (5) and Rehm and Cline (15). Coleoptile sections, 3 mm in length, were cut 3 mm from the tip of 3-d-old etiolated oat seedlings. Following removal of the leaves, four sections were suspended on a wire and then placed into the growth-measuring device. Constant temperature (25°C) was maintained during growth measurements.

The hollow core of coleoptiles of *A. fatua* had a slightly smaller diameter than that found in *A. sativa*. This made it impossible to place coleoptiles on the growth wire without damage to the tissue. Preliminary results indicated that coleoptiles of both species responded similarly to IAA in the straight growth test. Moreover, the dose-response curve for the inhibition of auxin-induced growth by fatty acids were similar for both wild oat and cultivated oat coleoptiles (unpublished results).

Extraction and Quantitation of Endogenous Fatty Acids. Dehulled seeds were frozen with liquid N_2 and quickly pulverized. The seed material was transferred to a 30-ml centrifuge tube containing 20 ml boiling isopropanol and stoppered immediately. After 30 min, the material was homogenized in ice-cold methanol (20 ml) and chloroform (80 ml). The homogenate was stirred at 5°C for 4 h. The extract was filtered, and the filtrate was partitioned three times against 10 mM KH_2PO_4 at pH 2.0. The organic phase was dried over anhydrous Na_2SO_4, and the solvents were removed under reduced pressure at 30°C to incipient dryness. The residue was resuspended in 2 ml hexane and charged onto a column of silicic acid (3 g) in hexane. The fraction containing free fatty acids was obtained following elution with 50 ml 15% (v/v) ethyl acetate in hexane.

Following evaporation of the solvent, the residue was redissolved in 2 ml diethyl ether and the fatty acid fraction was separated by the method of McCarthy and Duthie (14) on silicic acid treated with alkaline isopropanol. The fatty acids were eluted with 50 ml 2% (v/v) formic acid in diethyl ether followed by 50 ml diethyl ether. The ether was removed under reduced pressure and the residue was redissolved in CS_2 prior to GC analysis.

Quantitative estimates of the short chain fatty acids in seed extracts were achieved using a Varian model 3700[3] gas chromatograph equipped with a Varian CDS 111 integrator. The free fatty acids were chromatographed isothermally at 170°C on a glass column (183 × 0.2 cm i.d.) packed with 10% SP1200 and 1% H_3PO_4 on 80/100 Chromosorb W AW (Supelco). Carrier gas was N_2 with a flow rate of 30 cm^3 min^{-1}. All extractions were performed in triplicate. The extraction procedures were also performed in parallel on 10 mg pure pentanoic (C 5) acid and nonanoic (C 9) acid. Based on losses incurred on the pure acids, extraction efficiencies were about 50%. This figure, however, was not used in the quantiative estimates of the endogenous levels of free fatty acids.

All of the experiments examining the physiological and biochemical effects of short chain fatty acids employ NA. Similar results were obtained with other short chain fatty acids (C 6–C 10). All experiments reported here have been repeated at least three times with essentially identical results.

RESULTS

Characterization of Fatty Acid Inhibition of Seed Germination. Berrie *et al.* (3) presented data which represented the concentration of short chain fatty acids (C 6–C 11) that was required to cause 50% reduction in germination of nondormant wild oat seeds. While performing similar dose-response experiments, we observed curves that were very steep, and hence, had difficulty in defining a concentration that caused 50% inhibition of germination. Figure 1 illustrates a representative dose-response curve for the inhibition of nondormant wild oat seeds by NA. There is a striking increase of inhibitory activity between 7.5 and 10 mM. Whereas most

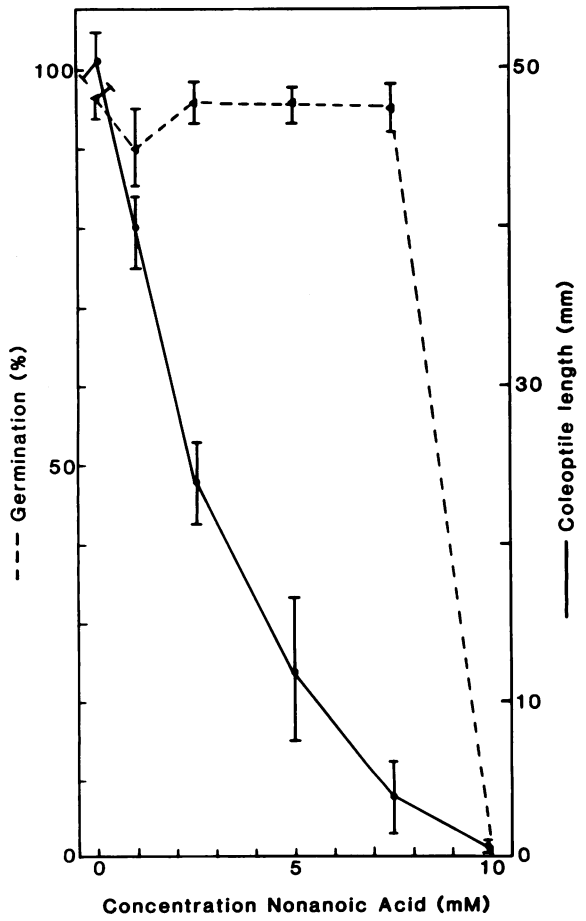

FIG. 1. Dose-response curve for the inhibition of germination (- - -) and coleoptile growth (——) by NA in nondormant wild oat seeds. Each value represents the mean of five replicates of 20 seeds each. Vertical bars represent SD.

[3] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

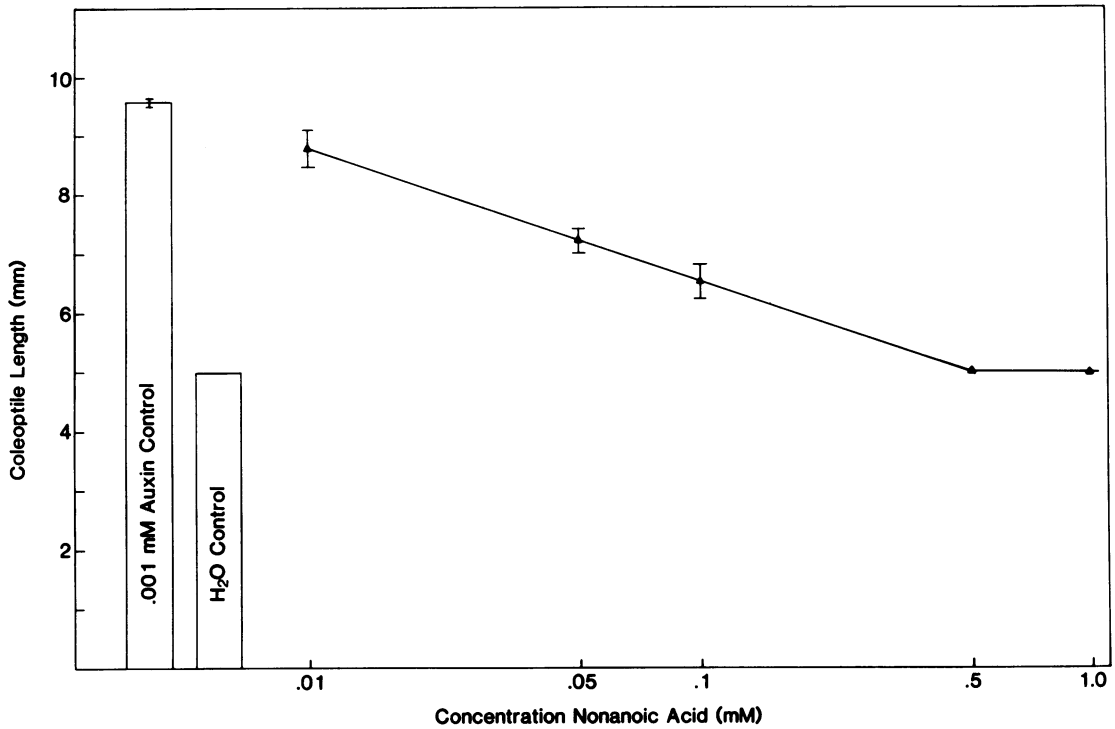

FIG. 2. The effect of NA on auxin-induced growth in excised wild oat coleoptiles. Coleoptile length was measured 24 h following initiation of treatment. Each treatment had three replicates of 10 coleoptiles each. Vertical bars represent SD.

hormone responses are proportional to the logarithm of the concentration, the inhibition of germination by NA exhibited a threshold effect. Similarly shaped dose-response curves have been reported for the inhibition of germination of seeds in other species by short chain fatty acids (23, 24).

Stewart and Berrie (23) reported that the transition between germinability and nongerminability in lettuce seeds with increasing concentration of short chain fatty acids was essentially linear. This implied that, during the course of determining the dose-response relationship between short chain fatty acid concentration and the inhibition of seed germination, several concentrations were found that caused varying degrees of inhibition. However, we consistently observed either essentially 100% germination or 100% inhibition over a small range of concentrations.

Increasing concentrations of NA progressively reduced the growth of the coleoptile and roots of intact seedlings until all growth ceased and no germination occurred (Fig. 1). In contrast to the effect of NA on seed germination, the inhibition of coleoptile growth was logarithmic in nature. The concentration of NA that inhibited growth by 50% was about 2.8 mM.

Effect of Short Chain Fatty Acids on Coleoptile Growth. The inhibition of auxin-induced growth in excised coleoptiles by NA was examined to determine the nature of inhibition of growth (Fig. 2). The dose-response curve, like that of intact seedlings, is logarithmic. Excised coleoptiles were more sensitive than coleoptiles from intact seedlings to the inhibition of growth by nonanoic acid. The NA concentration for 50% inhibition of growth in excised coleoptiles was 50 μM in contrast to 2.8 mM for coleoptiles in intact seedlings (Figs. 1 and 2). This enhancement of sensitivity could be due to the different experimental conditions used in the performance of the two assays. When excised coleoptiles were floated on a test solution in the straight growth test, the constant agitation provided by the reciprocating shaker allowed for uniform and more efficient penetration of compounds over the entire surface of the coleoptile. Intact seedlings, on the other hand, were exposed to the test solution via direct contact on the portion of the seed and coleoptile that was against the wetted blotter paper. This probably reduced the amount of NA available for inhibition of coleoptile growth.

The lag period for the initiation of NA inhibition of coleoptile growth was determined by examining the kinetics of continuously monitored coleoptile growth in *A. sativa* (Fig. 3). The first perceptible change in the growth rate was observed within 5 minutes (Fig. 3). At the highest concentration (10 mM) of NA used, the coleoptiles were flaccid and began to shrink after about 2 h following the introduction of the acid. This indicated that the fatty acids might act at the membrane level.

Exogenous Fatty Acids and the Induction of Seed Dormancy. While exogenous fatty acids inhibit wild oat seed germination, it is essential to know if that inhibition represents the induction of dormancy or nonspecific toxic effects. We examined this possibility in two ways. First, it is well established that phytohormone treatments will break dormancy in wild oat seed, including gibberellins (6), cytokinins (18), and ethylene (1). Table I shows that no hormone treatment at concentrations which we found to be effective in overcoming dormancy (unpublished results) was able to overcome the inhibition of germination of wild oat seeds by 10 mM NA. Higher concentrations of hormones were equally ineffective (data not shown).

Simmonds and Simpson (20) have shown that a correlation exists between release of seed dormancy and increased activity of the PP pathway in a particular inbred line of *A. fatua*. The relative proportion of the PP pathway to the EMP pathway in respiratory glucose metabolism may therefore be a useful biochemical characteristic that distinguishes dormant and nondormant wild oat seeds. Thus, if the inhibition of seed germination by exogenous fatty acids respresents the induction of primary dormancy, it is reasonable to expect that nondormant seeds treated with NA would exhibit characteristics of glucose metabolism as dormant seeds, namely a greater degree of participation by the EMP pathway. One method that has been used to discern the relative contribution of the PP pathway and the EMP pathway to respiratory glucose metabolism is to measure the ratio of $^{14}CO_2$ produced when seeds are fed either [6-^{14}C]- or [1-^{14}C]glucose. A ratio

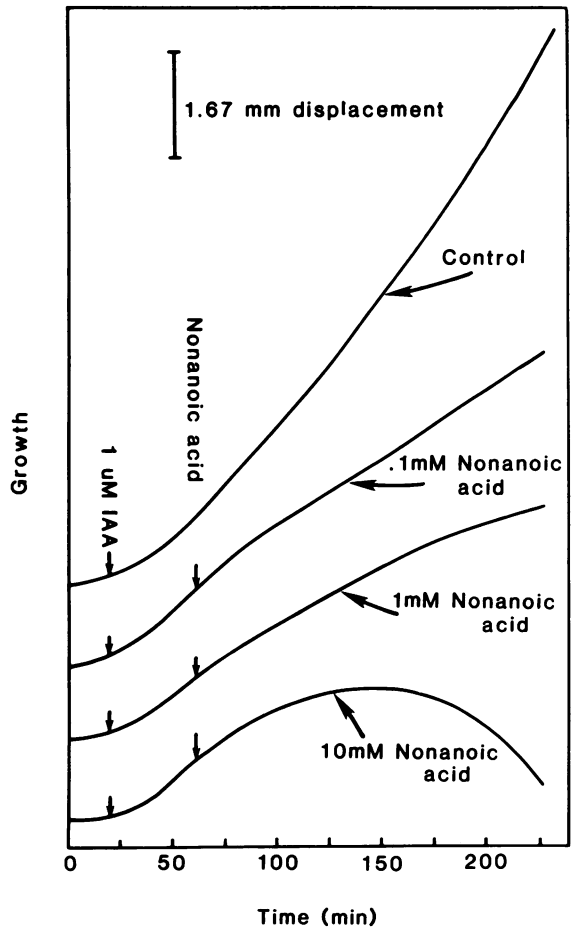

FIG. 3. The effect of NA on the continuous growth of excised coleoptiles of cultivated oat (*Avena sativa*). Figure represents the total length of four coleoptiles as a function of time. Growth curves were taken directly from recorder traces. First arrow represents the introduction of 1 μM IAA plus 0.1% Tween 20, and various concentrations of NA. All solutions contained 1 mM K_2SO_4 and $CaSO_4$ at pH 6.2.

Table I. *The Effect of Various Hormone Treatments on the Inhibition of Wild Oat Seed Germination by 10 mM Nonanoic Acid*

Percentage of germination is the mean of five replicates of 20 seeds each.

Treatment	Germination
	%
Control	97.5
NA (10 mM)	0
Gibberellin A_3 (0.1 mM)	100
NA + gibberellin A_3	0
Kinetin (0.01 mM)	98.6
NA + kinetin	0
Ethylene (100 μl/l)	100
NA + ethylene	0
NA + gibberellin A_3 + kinetin + ethylene	0

of dpm in $^{14}CO_2$ from [6-^{14}C]glucose to dpm in $^{14}CO_2$ from [1-^{14}C] glucose of unity indicates that glucose is metabolized mainly via the EMP pathway, while lower values indicate increased participation of the PP pathway. The effect of 10 mM NA on the C 6/C 1 ratio is shown in Table II. As expected, dormant embyros had a higher C 6/C 1 ratio than nondormant embyros. These results are consistent with those found previously for wild oat (20). However, treatment of nondormant embyros with 10 mM NA did not increase the C 6/C 1 ratio to the same level as that of dormant embyros. Moreover, NA treatment also decreased the amount of $^{14}CO_2$ produced when fed either [6-^{14}C]- or [1-^{14}C] glucose, indicating a disruption of cellular activities.

The Effect of Exogenous Fatty Acids on Membrane Integrity. Exogenous volatile fatty acids increase the permeability of roots to cations (9–11). Moreover, the effect on permeability is immediate, as is a change in the fatty acid composition of membrane lipids (9). A rapid effect by exogenous NA on membrane structure/function could conceivably initiate deleterious effects on other metabolic activities (12, 22, 25). We used leakage of amino acids and proteins from seeds into the imbibition solution as a measure of membrane integrity. Figure 4 shows the effect of various concentrations of NA on the loss of ninhydrin-positive substances from seeds into the imbibition solution as determined by a colorimetric assay. Clearly, treatment with NA caused an increase in the leakage of ninhydrin-positive substances from both dormant and nondormant seeds into the imbibition solution.

The dose-response data exhibit a log-linear relationship. More importantly, significant increases in the leakage of ninhydrin-positive substances occurred at concentrations of NA below that required for the inhibition of germination. The leakage by dormant control seeds was less than nondormant seeds imbibed in the presence of NA. This is another indication that the inhibition of germination by short chain fatty acids has a different biochemical basis than natural dormancy. The loss of membrane integrity has also been associated with the onset of thermal inhibition of seed germination in a variety of weed species including wild oat (8) and with the loss of viability in aging rice seeds (4).

The Effect of Reduced Pressures on the Endogenous Levels of Short Chain Fatty Acids in Seeds. If the process of afterripening involves the vaporization of endogenous short chain fatty acids, then the release from dormancy should be hastened considerably by subjecting dormant seeds to reduced pressures. Two hundred dehulled dormant and nondormant seeds were placed in a lyophilizer at 5 μm Hg. Both the vacuum-treated seeds and the controls (1 atm) were maintained at 22 to 24°C. After 1 week, 100 seeds from each treatment were imbibed and counted for germination. The endogenous fatty acid (C 6–C 10) content of the remaining 100 seeds was determined. Table III shows that treatment of dormant seeds with vacuum for 1 week did not break dormancy, but also had no effect on the germinability of nondormant seeds. However, treatment with vacuum did not appreciably alter the endogenous levels of short chain fatty acids.

Berrie *et al.* (2) detected a decline in the amount of individual short chain fatty acids (C 6–C 10) in seeds of *A. fatua* by as much as 150-fold during afterripening. In contrast, we observed no correlation between short chain fatty acid content and dormancy in wild oat seeds. The levels of hexanoic (C 6), heptanoic (C 7), and octanoic (C 8) acids did not decline during afterripening. The level of hexanoic acid increased substantially during afterripening, while the amount of nonanoic (C 9) acid exhibited a modest decline of about 20% during afterripening (Table III). No decanoic (C 10) acid was detected in either dormant or nondormant seeds. This is in sharp contrast to the observation by Berrie *et al.* (2) who found that decanoic acid formed the major portion of the total short chain fatty acid fraction (C 6–C 10).

It can be argued that since the embryonic axis is the organ which grows and results in germination, it is here where important changes in the short chain fatty acid content must occur. Therefore, we examined the short chain fatty acid content of embryonic axes excised from 100 dormant or nondormant seeds. The isolated axes were free of any adhering endosperm or aleurone cells. However, about 5% to 10% of the scutellum still remained. While the axes contributed to no more than 1.4% of the total weight of the dehulled seed, they contained about 20% of the seeds' total short chain fatty acid content. However, no differences in the qualitative pattern of the short chain fatty acid content between

Table II. *Effect of Nonanoic Acid on Respiratory Glucose Metabolism and the Glucose C 6/C 1 Ratio in Excised Embryos of Avena fatua during the First 12 h from Start of Imbibition*

Each treatment had three replicates of 100 excised embryos.

Treatment	$^{14}CO_2$ Radioactivity		Radiochemical Yield[a]		C 6/C 1[b]
	[1-^{14}C]Glucose (± SD)	[6-^{14}C]Glucose (± SD)	[1-^{14}C]Glucose	[6-^{14}C]Glucose	
	dpm		%		*ratio*
Dormant control	26,412 (230)	24,634 (140)	2.89	2.64	0.93
Dormant + 10 mM NA	5,600 (132)	4,017 (93)	0.56	0.47	0.84
Nondormant control	32,214 (432)	20,604 (102)	3.22	2.42	0.75
Nondormant + 10 mM NA	9,519 (78)	5,823 (56)	0.95	0.68	0.72

[a] Percentage of radioactivity (dpm) converted to $^{14}CO_2$ from [^{14}C]glucose.

[b] Per cent radiochemical yield of $^{14}CO_2$ from [6-^{14}C]glucose/per cent radiochemical yield of $^{14}CO_2$ from [1-^{14}C] glucose.

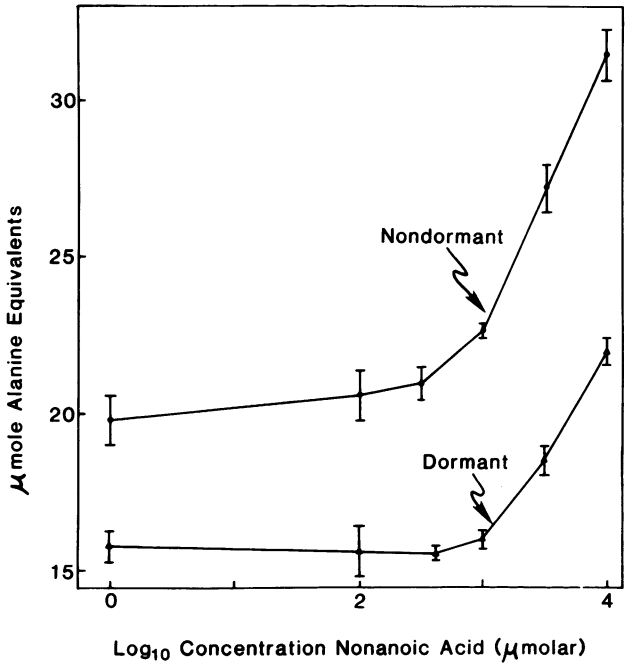

FIG. 4. The effect of various concentrations of NA on the leakage of ninhydrin-positive substances from seeds into the imbibition solution. Each point represents the mean of three replicates of 50 seeds each. Vertical bars indicate SD.

whole seeds and the embryonic axes were observed (data not shown). Thus, there was no correlation between dormancy and the short chain fatty acid content of the embryonic axes.

DISCUSSION

The Mechanism of Short Chain Fatty Acid Inhibition of Germination. Any critical evaluation of the hypothesis that germination inhibitors are responsible for the induction and maintenance of seed dormancy must include a comparison between the physiological and biochemical states of seeds in natural dormancy and nondormant seeds in which germination has been inhibited by exogenous substances. In our experiments, exogenous NA did not return nondormant seeds to a biochemical and physiological state similar to dormant seeds (Tables I and II). Seeds in which germination was inhibited with exogenous NA were not viable according to the tetrazolium test. Untreated dormant seeds, both dry and soaked in the presence of H_2O for 4 d were able to reduce 2,3,5-triphenyltetrazolium to triphenylformazan (unpublished results). Repeated efforts to find a concentration of NA which inhibited germination but which did not destroy viability failed. This indicates that the mechanisms of inhibition of germination are different in primary dormancy and nondormant seeds treated with NA. Furthermore, the NA-induced inhibition of germination cannot be ascribed to the induction of some form of secondary dormancy either, because that would imply the maintenance of viability in spite of the loss of the ability to germinate.

Table III. *The Effect of Vacuum (5 μm Hg for 1 Week) on the Germinability and the Short Chain Fatty Acid Content of Dormant and Nondormant Wild Oat Seeds*

Figures represent the mean of three replicates.

Acid	Amount of Fatty Acid			
	Nondormant control (95%)[a]	Nondormant vacuum (94%)	Dormant control (10%)	Dormant vacuum (8%)
	ng grain^{-1}			
Hexanoic (C 6)	340 (21)[b]	380 (32)	ND[c]	ND
Heptanoic (C 7)	180 (16)	239 (41)	95 (13)	123 (17)
Octanoic (C 8)	1067 (61)	1180 (52)	940 (58)	1022 (56)
Nonanoic (C 9)	490 (12)	505 (18)	603 (10)	582 (8)
Decanoic (C 10)	ND	ND	ND	ND

[a] Per cent germination of 100 seeds.

[b] SD in parentheses.

[c] ND, none detected.

Exogenous NA inhibited glucose oxidation, suggesting disruption of metabolism (Table II). We have also found that, when NA was included in the imbibition solution, the seeds' ability to incorporate [^{35}S]methionine into protein and [^{3}H]glycerol into all major lipid fractions was impaired. However, significant inhibition of these biosynthetic reactions occurred only 24 h after the start of imbibition, whereas germination of nondormant control seeds was observed after 12 h (unpublished results). The biochemical basis for the inhibition of germination by exogenous short chain fatty acids cannot be due to disruption of these basic cellular processes.

We observed a progressive inhibition of the coleoptile and roots with increasingly higher doses of NA, culminating ultimately in the inhibition of germination (Fig. 1). It is possible that exogenous NA does not specifically and reversibly block events leading to

germination as in the dormancy mechanism, but instead, progressively reduces growth of the embryo until germination becomes impossible. This scenario explains the threshold dose-response curve for the inhibition of germination by exogenous short chain fatty acids (Fig. 1). In lettuce, a similar relationship between the dose-response curves for the inhibition of root growth and germination by short chain fatty acids has been observed, although the author did not specifically link the two phenomena (24).

Growth of the embryo is the sum of cell division and cell enlargement. In germinating cereal seeds, it appears that elongation initially contributes more to growth than cell division (7). Cell enlargement depends on the maintenance of turgor pressure, which in turn is dependent on proper membrane structure/function. Thus, membrane perturbations can lead to rapid effects on growth (12, 13, 25). We observed that exogenous NA had a deleterious effect on membrane integrity in imbibing seeds (Fig. 4). Moreover, the growth of excised coleoptiles was inhibited within 5 min following application of NA (Fig. 3), which is consistent with our proposal that fatty acids inhibit growth and, therefore, germination by initially disrupting membrane activities. Also, membrane perturbations result in alterations in intracellular pH and ion fluxes (12, 22). These changes could lead to effects on enzyme activity, protein synthesis, or cell division (13, 25). In this view, the effect on growth is immediate, but the initial effect on membrane structure/function also leads to secondary effects on other metabolic activities, leading ultimately to the death of the seeds.

Vaporization of Endogenous Short Chain Fatty Acids from Seeds as the Mechanism Underlying Afterripening. We showed that afterripening was not hastened by maintaining dormant seeds at reduced pressures (5 μm Hg). However, such treatments had no effect on the volatile fatty acid content (Table III). This suggests that the endogenous fatty acids are not available for evaporation. When 50 mg pure NA were placed in a 10-ml beaker and subjected to a 5 μm Hg vacuum, all of the acid was evaporated within 4 h. However, very little sodium salt of NA vaporized even after 1 week under the same conditions (data not shown). The precise subcellular location of free short chain fatty acids is not known. Moreover, it is an open question whether short chain fatty acids exist in wild oat seeds as undissociated acids or as salts. Nevertheless, one explanation for the failure to lower the endogenous levels of short chain fatty acids under a vacuum is that the acids exist as salts. If this is so, the hypothesis that vaporization of short chain fatty acids is the mechanism underlying afterripening becomes much less likely.

We did not observe the correlation between endogenous levels of short chain fatty acids (C 6– C 10) in seeds or embryonic axes and dormancy as was reported by Berrie *et al.* (2). While these discrepancies are difficult to explain, it is important to note that the seed stocks used in both studies were genetically heterogeneous, and from different areas with divergent environments. This could possibly account for the observed differences and vividly points out the dangers associated with the development of hypotheses based on experiments on plant material with such a heterogeneous genetic background as found in wild oats (21). In total, we believe the results presented in this paper seriously question the postulated role of short chain fatty acids in the induction and maintenance of seed dormancy in *A. fatua.*

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