STUDIES ON THE INDUCTION AND RELEASE
OF SEED DORMANCY IN WILD OATS
(AVENA FATUA L.)

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ABSTRACT

The induction and release of secondary dormancy by anaerobiosis in genetically pure dormant (AN-51, Mont 73) and nondormant (CS-40, SH-430) lines of *Avena fatua* L. and the role of alternative respiration in the regulation of its primary and secondary dormancy were studied. These lines differed with regard to the optimal period of anaerobiosis necessary for induction of dormancy and/or the degree (% of seeds acquiring dormancy) and duration of dormancy induced. Secondary dormancy could be induced more effectively in after-ripened seeds of dormant lines than in nondormant lines where only a short-term dormancy could be induced (in 5-7 week old seeds). As with primary dormancy, wild oat biotypes exhibit genetic variability in their secondary dormancy behaviour and factors such as temperature can modify the expression of this trait. The germination stimulants kinetin, isopentenyl adenine, sodium azide, potassium nitrate and ethanol, which break primary dormancy in wild oats, stimulated germination of secondarily dormant seeds (line AN-51). Since these chemicals are structurally diverse, primary and secondary dormancies appear to be regulated by similar mechanism(s).

Salicylhydroxamate (SHAM), an inhibitor of alternative respiration, did not inhibit: 1. the respiration of embryos excised from after-ripened or secondarily dormant seeds, 2. the spontaneous release of secondary dormancy in nondormant lines or 3. the release of secondary dormancy by a variety of chemicals (except azide), suggesting that alternative respiration is not involved in the induction or release of secondary dormancy.

Azide and cyanide released seed dormancy at similar concentrations.
and treatment durations. While cyanide released primary dormancy in seeds with little after-ripening, azide was effective only in secondarily dormant seeds or seeds with more extensive after-ripening. Both inhibitors stimulated seed respiration to a similar extent. The release of dormancy by cyanide was always preceded by respiratory stimulation, but the latter appeared to be independent of germination.

SHAM inhibited both the release of seed dormancy and the stimulation of seed respiration by azide but not by cyanide. Respiration was inhibited only when SHAM was applied concurrently with azide. When applied subsequent to azide treatment, SHAM had no effect. The respiration of seed pre-treated with azide and cyanide was insensitive to SHAM and therefore cannot be alternative.

Studies were performed to determine the effect of pH on the stimulation of germination and respiration by cytochrome oxidase inhibitors. Although pH had little effect on seed respiration and germination in controls and in the presence of cyanide, it strongly affected the activity of azide. At pH 5, 1 mM azide inhibited both seed respiration and germination whereas at pH 7 it stimulated both. SHAM at pH 7 did not affect the stimulation of respiration by azide, but inhibited it in the unbuffered system and at pH 5. Thus, SHAM appears to alter azide activity by lowering pH, increasing the concentration of undissociated (active) azide, which then completely inhibits cytochrome oxidase and consequently, seed respiration and germination. The release of dormancy and the stimulation of respiration by cyanide and azide do not appear to be related to the inhibition of cytochrome-mediated respiration or the stimulation of alternative respiration.
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INTRODUCTION AND LITERATURE REVIEW

I. INTRODUCTION

Seed dormancy*, a widespread phenomenon in the plant kingdom, is ecologically advantageous for several reasons. Firstly, germination can be controlled so as to limit emergence to times most conducive to seedling establishment. Secondly, a dormancy mechanism can function to secure a suitable place for germination (i.e. seeds requiring light for dormancy release will germinate only in the top few millimeters of soil). Lastly, seed dormancy prevents premature germination (i.e. while seeds are still on the maternal plant; Bewley and Black, 1985).

Seed dormancy is of great agricultural significance. Although most crop species produce nondormant seed (because of human selection for prompt and uniform seed germination), weed species display a wide diversity in types of dormancy. Variability in weed seed germinability ensures germination over an extended period making control difficult.

*For this study the definition of seed dormancy given by Simpson (1978) will be used: "dormancy is that state in which a seed fails to resume growth when it is rehydrated in an environment that will support normal germination and seedling growth of apparently identical but nondormant seeds of the same species".
Seed dormancy can be divided into two categories: 1. Embryo dormancy and 2. Coat-imposed dormancy. In embryo dormancy, the control mechanism resides within the embryo which fails to germinate when removed from the rest of the seed tissue. Coat-imposed dormancy is maintained by a hard, impervious seed coat which encloses the embryo and endosperm (Bewley and Black, 1985). The control of embryo dormancy can be a function of: 1. the presence of germination inhibitors, 2. the existence of a biochemical block that prevents or is necessary for germination or 3. physiological immaturity of the embryo. Coat-imposed dormancy may be manifested by: 1. mechanical restraint (i.e. "hard seeds"), 2. interference with water or oxygen uptake or 3. the presence of germination inhibitors within the seed coat (Bewley and Black, 1982).

Both the acquisition and subsequent release of dormancy are controlled by an interaction between genetic and environmental factors. Environmental factors regulating the acquisition of seed dormancy include light, temperature and oxygen availability. Time is important in the release of seed dormancy during dry storage (after-ripening) (Bewley and Black, 1985).

B. SEED DORMANCY IN WILD OAT.

Wild oats (Avena fatua L.) is an important weed of cereal and oilseed crops around the world. Originating in Eurasia (Frankton and Mulligan, 1970), its range now includes several European countries, North Africa, Australia, New Zealand and North America.
Its distribution in Canada is predominantly western, being most abundant in the three prairie provinces and the Peace River region of B.C. (Sharma and Vanden Born, 1978). In terms of crop losses and weed control costs, *A. fatua* is Canada's most detrimental weed species (Alex, 1982). In 1978, estimated losses due to wild oats in the grain growing areas of Canada exceeded $280 million (Alex, 1982).

The persistence of wild oats as a weed largely depends on the ability of its seed to remain dormant in the soil for varying periods. Different biotypes of wild oats produce seed with dormancy ranging from a few weeks up to 5 years (Naylor and Jana, 1976; Sharma and Vanden Born, 1978). In addition, nondormant (through after-ripening) seeds are able to acquire secondary dormancy under adverse environmental conditions (Hay and Cumming, 1959). An understanding of the physiological basis of seed dormancy is essential for the development of more effective control measures for this weed.

Wild oats provide an excellent system for the study of seed dormancy. Genetically pure lines exhibiting different seed dormancy behavior have been isolated from field populations. By raising these lines under controlled growth conditions it is possible to compare genetically nondormant and dormant seeds in the absence of confounding environmental effects on dormancy development. The loss of wild oats seed dormancy through after-ripening and the ability of the after-ripened seeds to acquire secondary dormancy enables comparison of different states of dormancy within a line thus circumventing genetic differences that could hamper such a study. Dormancy in wild
oats is believed to be embryonic in origin. Embryos excised from freshly harvested dormant wild oats seeds failed to germinate when imbibed on water or nutrient media (Naylor and Simpson, 1961; Simpson, 1965) while embryos from nondormant seeds germinated under these conditions. As dormant seeds after-ripened, the percentage germination of excised embryos increased. Addition of GA$_3$ (gibberellic acid-3) to the medium overcame embryo dormancy (Simpson, 1965).

Genetic (Naylor and Jana, 1976; Jana et al., 1979) and environmental influences on seed dormancy in wild oats have been well documented. Environmental conditions during seed maturation and seed germination alter the expression of dormancy in wild oats (Sawhney and Naylor, 1979, 1980; Peters, 1982). Sawhney and Naylor (1979) found that high temperature during seed maturation greatly reduced the duration of primary dormancy in some biotypes of wild oats. The loss of primary dormancy in imbibed seeds however, is favored by low incubation temperatures (Naylor and Fedec, 1978). Mature seeds of genetically pure lines differ in their germination response to temperature during imbibition (Naylor and Fedec, 1978). Water stress during seed maturation also affects dormancy expression in wild oats. Peters (1982) found that water stress, imposed from the time of panicle emergence to the completion of ripening, increased the germination percentage of mature seeds, indicating that the duration of dormancy was much shorter in seeds from water stressed plants compared to those from nonstressed plants.

The biochemical basis of seed dormancy in wild oats is not known.
Much of the work involving the physiology of dormancy release is of an empirical nature and has contributed little to a general understanding of the mechanism of dormancy in this species. GA$_3$ is a potent dormancy-breaking treatment for wild oats seed (Naylor and Simpson, 1961b). Consequently, a mechanism whereby dormancy is regulated by this compound has been sought. Naylor and Simpson (1961b) observed two distinct concentration optima for GA$_3$ in the presence and absence of sucrose. Alone, 50ppm GA$_3$ was required to release dormancy in freshly harvested seed but when given in combination with sucrose, 0.5 ppm GA$_3$ released dormancy. This bimodal concentration optima was explained by a dual mode of action of GA$_3$; high GA$_3$ concentrations are required to stimulate starch breakdown in endosperm whereas when applied with sucrose, a lower GA$_3$ concentration was adequate to enable sugar utilization and germination by the embryo. Thus, while GA$_3$ was required for endosperm mobilization, the release of dormancy could be elicited by GA$_3$ in the absence of endosperm breakdown. Metzger (1983a), however, found no difference in endogenous GA$_3$ levels in genetically dormant and nondormant lines of wild oats. Furthermore, no significant change in gibberellic acid level occurred during the loss of dormancy through after-ripening (Taylor and Simpson, 1980). Thus, dormancy in wild oats cannot be explained simply in terms of endogenous GA$_3$ level.

C. STIMULATION OF WILD OAT SEED GERMINATION BY CHEMICALS

Adkins and Ross (1981) found that the growth regulator
ethylene could stimulate germination in partially after-ripened seeds of wild oats. They did not however find any relationship between endogenous ethylene production and germinability.

Roberts and Smith (1977) proposed that nitrate and nitrite ions, both of which promote wild oats seed germination (Johnson, 1935; Adkins et al., 1984b), act to promote the reoxidation of NADPH to NADP via the oxidative pentose phosphate pathway (PPP) which in turn promotes germination. They suggested that any compound stimulating the PPP will release seed dormancy. Azide, an inhibitor of cytochrome oxidase, is known to release dormancy in wild oats (Fay and Gorecki, 1978). Roberts and Smith (1977) suggested that azide promotes seed germination by stimulating the PPP. In light of more recent research this hypothesis has however, been dismissed. No relationship between the levels of PPP dehydrogenases (Upadhyaya et al., 1981) or PPP activity measured by the $C_6/C_1$ ratio technique (Fuerst et al., 1983) and dormancy status could be found. Similarly the release of dormancy by GA$_3$ did not result in the concomitant rise in the activity of the PPP as Robert's hypothesis would require. This hypothesis is therefore not substantiated in wild oats. Recently Adkins et al. (1984c) have suggested that nitrate and nitrite may release seed dormancy in wild oats by promoting glycolysis and/or the Kreb's cycle by enhancing the rate of reoxidation of NADH. Evidence supporting this hypothesis is also lacking.
Anesthetics are another class of compounds known to release seed dormancy in several species (Taylorson and Hendricks, 1979) including wild oats (Upadhyaya, unpublished data; Adkins et al., 1984a,d). Hendricks and Taylorson (1980) suggested that ethanol and some other anesthetics release seed dormancy by increasing cell membrane permeability. The bases of their conclusion were: 1. several metabolically inert anesthetics (i.e. chloroform, ethyl ether) released seed dormancy, suggesting that these compounds release dormancy via their action on cell membranes rather than as respiratory substrates, and 2. application of hydrostatic pressure (known to decrease cell membrane permeability, Hendricks and Taylorson, 1980) to seeds reversed the effect of applied anesthetics (Hendricks and Taylorson, 1980). Adkins et al. (1984a) found that ethanol, acetaldehyde and to a lesser extent, procaine, chloralhydrate and methanol stimulated germination of dormant wild oats seeds. Since the two most effective anesthetics (ethanol and acetaldehyde) are metabolically active, it was suggested that the promotion of germination by these compounds may be due to their role as respiratory substrates rather than by the virtue of their effects on cell membranes (Adkins et al., 1984d). Accordingly, application of ethanol to seeds is thought to enhance the flux of metabolites through the Kreb's cycle, which the authors propose regulates seed dormancy in wild oats.

Substituted phthalimides have also been shown to release dormancy in several weed species including wild oats (Metzger, 1983b; Upadhyaya et al., 1986). It is believed that these compounds act like GA₃ in this regard.
D. SEED DORMANCY AND ALTERNATIVE RESPIRATION

Recent studies on the mechanism of the release of seed dormancy using respiratory inhibitors have implicated alternative (cyanide-insensitive) respiration as being important in its regulation. Before discussing the evidence for this theory the biochemistry and physiology of this pathway will be briefly reviewed.

1. A biochemical overview of the alternative pathway.

The occurrence of CN-resistant respiration in plant tissue has been observed since the early 1930's (Henry and Nyns, 1975). Although early classical studies of alternative respiration involved almost exclusively members of the Araceae, the phenomenon of CN-resistance is now known to be widespread in the plant kingdom (Henry and Nyns, 1975).

The cellular location of alternative respiration has been ascribed to the mitochondria. James and Beevers (1950) reported that oxygen uptake in mitochondria isolated from CN-resistant spadix tissue of *Arum maculatum* was CN-resistant. Subsequently numerous studies have reported similar results in a variety of tissues (Solomos, 1977). Recent research indicates that components of the alternative pathway are embedded in the inner mitochondrial membrane (Troostembergh and Nyns, 1976).

The discovery of selective inhibitors of the alternative pathway (hydroxamic acids, Schonbaum *et al.*, 1971; disulfiram, Grovers and Laties, 1981) has greatly advanced our knowledge of its biochemical
Fig. 1 The cytochrome (A) and alternative (B) pathways.
X = alternative oxidase. Midpoint redox potentials are given in brackets. (from: Lambers, 1980).
and physiological nature. Using these inhibitors it has been demonstrated that when the cytochrome pathway is blocked (i.e. in the presence of cyanide) the contribution of alternative respiration increases. This suggests that the two pathways share some common components with the alternative pathway providing a "detour" for electrons in the presence of inhibitors of cytochrome-mediated respiration (Lambers, 1980).

Bendall and Bonner (1971) demonstrated that CN-resistant mitochondria possess two oxidases - one being CN-resistant (alternative oxidase) and the other CN-sensitive (cytochrome oxidase). The alternative respiratory pathway was determined to branch from the main respiratory pathway before the b-cytochromes. It is now generally accepted that the branch point occurs at ubiquinone (Lambers, 1980, Fig. 1). While the exact nature of the alternative oxidase is not known, current evidence suggests that: 1. it is a quinoloxidoreductase (Laties, 1982), 2. its affinity for oxygen is about 10 times lower than that of cytochrome oxidase (Solomos, 1977), 3. it is non-phosphorylating (Akimenko et al., 1979) and 4. its final end-product is water (Rich et al., 1978)

2. The physiological significance of alternative respiration.

The wide-spread occurrence of CN-resistant respiration has enticed scientists to search for the physiological significance of this pathway. Because it is non-phosphorylating, oxidative energy can be released in the form of heat. In Arum spadix tissue, where the capacity of the alternative pathway is 3-4 times that of the
cytochrome pathway, the temperature of the spadix tissue may be 20°C higher than ambient temperature when the alternative pathway is fully operative (Lambers, 1980). Meeuse (1975) suggests that this rise in temperature volatilizes compounds which attract insect pollinators. Similarly, in *Symlocarpus*, heat production *via* the alternative pathway enables floral development and pollination to occur at sub-freezing temperatures (Meeuse, 1975). Nevertheless all tissues exhibiting CN-resistance are not thermogenic.

Solomos (1977) suggested that alternative respiration may be involved in the climacteric and ripening process of certain fruits. Cyanide was found to be as effective as ethylene in initiating the climacteric of some fruits. In the same vein, ethylene-induced respiration was found to be CN-resistant (Solomos and Laties, 1974). However, upon further examination using selective inhibitors of the alternative pathway, it was found that even at the climacteric peak, no alternative respiration was present in ripening avocados or bananas (Theologis and Laties, 1978b). Similarly, infiltrating pear fruits with SHAM (salicylhydroxamic acid - an inhibitor of alternative respiration; Schonbaum *et al.*, 1971) did not prevent the climacteric from occurring (Janes and Frenkel, 1978). Laties (1982) concluded that the climacteric cannot be explained in terms of the alternative pathway and warned against dogmatically assuming that CN-resistance automatically implies the *in vivo* functioning of the alternative pathway in the absence of cytochrome inhibitors.

Alternative respiration has been measured in the roots of many species. It has been implicated as having a role in the regulation
of anion uptake, flood tolerance and in providing a path for oxidation of excess substrates when storage capacity is exceeded (Latties, 1982). Conclusive evidence supporting these hypotheses has yet to be reported.

The alternative pathway may have a role in protecting some species from chilling injury (Yoshida and Tagawa, 1979). When chill-sensitive Cornus callus tissue was subjected to chilling stress, one or more of the components of the cytochrome pathway embedded in the inner mitochondrial membrane was disrupted, resulting in the diversion of electrons through the alternative pathway. This enabled ATP production to continue, although the respiratory control ratio dropped sharply.

3. Regulation of seed dormancy and alternative respiration.

Alternative respiration has been implicated as being physiologically significant in the regulation of seed dormancy and germination in several species. Evidence suggesting this role has been circumstantial. Since cyanide and other inhibitors of cytochrome-mediated electron transport are ineffective at completely inhibiting seed respiration, it has been suggested that oxygen-uptake in the presence of these inhibitors is alternative (Yentur and Leopold, 1976). It has been proposed that cyanide increases the flux of electrons through the alternative pathway in seeds, promoting germination (Hendricks and Taylorson, 1972). Further evidence suggesting involvement of alternative respiration in germination
and/or release of dormancy has involved the use of inhibitors of this pathway. Yentur and Leopold (1976) found that SHAM inhibited seed germination in several species. Similarly, Yu et al. (1979) found that SHAM (10 mM) inhibited red light-induced, release of dormancy in lettuce seeds (var. Grand Rapids); KCN is known to release dormancy in these seeds (Taylorson and Hendricks, 1973). In these studies the hypothesis that electron flux through the alternative pathway is required for the release of seed dormancy was advanced.

Although numerous studies have shown alternative respiration to occur in late or post-germination stages of nondormant seeds (James and Spencer, 1979; Morohashi and Matsushima, 1983; Burguillo and Nicolas, 1977) little research has been performed which directly examines its occurrence during the release of seed dormancy. The role of cytochrome mediated and alternative respiration in the release of dormancy of cocklebur seeds has however, been examined. Ethylene (Esashi et al., 1979b), cyanide (Esashi et al., 1981a) and fluctuating temperature (Esashi et al., 1983), all of which stimulate germination in dormant cocklebur seeds, evoked either a concomitant increase in alternative respiration or an increase in the alternative/cytochrome respiration ratio. The latter was suggested to regulate cocklebur germination (Esashi et al., 1981b). Furthermore, the stimulatory effect of ethylene (which also increases alternative respiration in other systems, Solomos and Laties, 1975) on seed germination in cocklebur was negated by a simultaneous application of SHAM (Esashi et al., 1979b). It was proposed that ethylene acted on the seeds to
induce the alternative pathway which in turn promoted the release of
dormancy. SHAM application inhibited the induced alternative
respiration and thus germination.

Azide has been shown to release seed dormancy in wild oats (Fay
and Gorecki, 1978). Upadhyaya et al. (1982a) found that this release
of dormancy could be prevented by a simultaneous application of SHAM.
It was subsequently found that azide stimulated seed respiration well
in advance of germination and concurrent treatment of seeds with SHAM
resulted in an almost complete inhibition of respiration (Upadhyaya et
al., 1983). The authors suggested that SHAM-sensitive (alternative)
respiration may be associated with and may be necessary for the
stimulation of germination by azide. Conclusive evidence supporting
such an involvement of alternative respiration has not yet been
presented.

E. SECONDARY SEED DORMANCY.

Secondary dormancy can be induced in seeds of several species by a
variety of methods. For example, secondary dormancy has been induced
by far-red light in lettuce (Vidavar and Hsiao, 1975), elevated
temperatures in common ragweed (Baskin and Baskin, 1980), darkness in
Lamium amplexicaule (Taylorson and Hendricks, 1976) and anaerobiosis
in cocklebur (Esashi et al., 1978) and wild oats (Hay and Cumming,
1959) seeds. Very little is known about the regulation of secondary
seed dormancy or how it compares physiologically with primary
dormancy.
Esashi et al. (1981b) have proposed that secondary dormancy in cocklebur seeds results from their inability to perform alternative respiration. While secondarily dormant seeds in cocklebur were not responsive to cyanide alone, its application following pretreatment with ethylene increased both germination and the alternative/cytochrome respiration ratio. It was suggested that cyanide was ineffective at releasing dormancy since the seeds were unable to perform alternative respiration. When ethylene was applied prior to cyanide, the alternative pathway was induced, which was then promoted by subsequent cyanide treatment resulting in germination.

In wild oats, compounds such as nitrate, peroxide and GA$_3$ are known to promote germination of secondarily dormant seeds (Hay and Cumming, 1959). Since the work of Hay and Cumming (1959), several genetically pure lines of wild oats differing in their dormancy behavior have been characterized and used in the investigation of primary dormancy (Fuerst et al., 1983; Sawhney and Naylor, 1980; Naylor and Fedec, 1978). Whether these lines differ in their ability to acquire secondary dormancy is not known. Furthermore, several additional chemicals have been identified which stimulate germination in seed of several weeds including wild oats (Adkins et al., 1984a,b; Esashi et al., 1978; Fay and Gorecki, 1978; Roberts and Smith, 1977; Upadhyaya et al., 1982a). These stimulants and pure lines can be useful tools in the investigation of the induction and release of secondary dormancy in wild oats.
F. Objectives.

The objectives of this study were to determine:

1. if genetically pure lines of wild oats differ with regard to the induction of secondary dormancy.
2. how temperature affects the induction of secondary dormancy.
3. whether primary and secondary dormancies differ in response to a variety of unrelated germination stimulants.
4. the involvement of alternative (SHAM-sensitive) respiration in the induction and release of secondary dormancy.
5. the similarities and differences of azide and cyanide-induced respiration and germination.
6. the relationship between cyanide-induced seed respiration and the release of dormancy by this compound.
7. the possible involvement of alternative respiration in the stimulation of respiration and germination by azide and cyanide.
8. the effects of pH on the action of respiratory inhibitors on wild oats seeds.
MATERIALS AND METHODS

A. Seed source.

In order to minimize extraneous variation in seed dormancy behavior, genetically pure lines of wild oats were used in all experiments. Two dormant (AN-51 and Mont 73) and two nondormant (SH-430 and CS-40) lines were used. Under our conditions, AN-51 and Mont 73 remained dormant for 6-12 months after harvest while SH-430 and CS-40 germinated at a rate of 100% after 2-3 weeks of after-ripening. Environmental factors (temperature and moisture levels) are known to effect the development of seed dormancy during seed maturation (Sawhney and Naylor, 1979, 1980). For this reason all plants raised for seed production were grown under controlled conditions in growth cabinets. Plants were grown in 2.7 L pots (3 plants/pot) in a 4:1 soil:peat mix (with 20-20-20 fertilizer) at a constant temperature of 20 C and a 16 h photoperiod with 200 W m\(^{-2}\) light intensity (Sylvania, VHO, cool-white fluorescent tubes supplemented with incandescent bulbs). Plants were fertilized with 100 mL pot\(^{-1}\) of 3 g L\(^{-1}\) Peters 20:20:20 (%NPK plus Mg, Fe, Mn, Zn, Cu, B and Mo) fertilizer at the flag-leaf stage. Seeds were harvested at maturity and used either within 4-8 weeks or were allowed to after-ripen for 1-2 years in the dark at about 25 C. Seeds were dehulled prior to use in all experiments.
B. The induction of secondary dormancy.

Secondary dormancy was induced by immersing after-ripened seeds in deaerated water (approximately 50 uM dissolved oxygen) in sealed Erlenmeyer flasks at 25 C in the dark for various durations (as specified). Deaeration was accomplished by boiling distilled water and allowing it to cool to the desired temperature in an air-sealed system which permitted the intake of boiled water to fill the air space produced on cooling. In experiments involving the effect of anaerobiosis temperature on the induction of secondary dormancy, dormancy was induced at 15, 20, 25 and 30 C. Upon completion of anaerobiosis, seeds were removed from the flasks and their germination monitored under aerobic conditions (25 C, in darkness) in 10 cm Petri dishes (10 seeds/dish) lined with two Whatman No. 1 filter disks wetted with 5 mL of water. Seeds were treated with Captan (50% wettable powder) prior to placement in the dishes. Seeds used to investigate the effect of pre-imbibition on the induction of secondary dormancy were imbibed for the specified periods under aerobic conditions (at 25 C) and then transferred to Erlenmeyer flasks for anaerobiosis.

C. Germination studies.

Seeds were placed embryo side-up in 10 cm Petri plates (10 seeds/plate) lined with two Whatman No. 1 filter disks wetted with
5 mL water or the indicated treatment solution. Chemicals were dissolved in distilled water or buffer (50 mM sodium phosphate or citrate-phosphate buffer) of specified pH. Buffers were prepared according to Gomori (1955) and diluted four fold; the buffering capacity was found to be adequate for all experiments. In experiments where KCN was used, Petri plates were sealed with a strip of Parafilm to minimize the escape of cyanide as gaseous HCN. Plates were stored in a saturated atmosphere at 25 °C in the dark. Germination (protrusion of the coleorhiza through the testa) was recorded at specified intervals. Seed viability was determined by percentage germination on addition of 1 mL of 1.4 mM GA₃ per dish at the completion of the experiment. Seed viability generally exceeded 90%. Experiments were performed with either 3 or 4 replicates.

D. Oxygen uptake measurements.

1. **Intact seeds:** Oxygen-uptake was monitored polarographically using a Clark-type oxygen electrode and a YSI Model 30 oxygen meter (Yellow Springs Instruments Co., Inc., Yellow Springs, Ohio). Upon completion of the incubation treatment in Petri dishes, seeds were rinsed three times with distilled water suspended in 5 mL of oxygen-saturated phosphate buffer (50 mM, pH 6.9) and oxygen consumption was recorded for 7-10 minutes. Two or 3 replicates (of 10 seeds each) were used in each experiment.
2. Excised embryos

i. Oxygen uptake: Embryo sections (embryo and adhering pericarp and testa) were excised from seed imbibed on water under a dissecting microscope. Excised embryos were gently vortexed to remove adhereing starch grains and then suspended in 3 mL phosphate buffer (50 mM, pH 6.9) with or without 5 mM SHAM. To determine respiratory components, 10 μL of concentrated NaN₃ or KCN solution was injected directly into the oxygen uptake vessel (to obtain desired inhibitor concentration) after a linear rate of oxygen consumption had been established. All experiments were performed using 3 replicates of 10 embryos each.

ii. Calculation of respiratory components: Respiratory components were determined from oxygen uptake data by the method of Theologis and Laties (1978a) for intact tissue. This method has been adapted from the analysis of respiratory components for isolated mitochondria (Bahr and Bonner, 1973). Here the respiration is described by the equation:

$$V_t = p.(g) + V_{cyt} + V_{res}$$

where: $V_t$ = total respiration rate

$V_{cyt}$ = cytochrome-mediated respiration
(gi) = maximal possible contribution of the alternative pathway

p = the fraction of the alternative pathway which is operative at any given time and therefore p(gi) represents the actual rate of alternative respiration ($V_{alt}$)

$V_{res}$ = residual respiration (that portion of total respiration that is insensitive to both inhibitors of cytochrome-mediated and alternative respiration).

Respiratory components can be determined in both the presence and the absence of cytochrome oxidase inhibitors - in both cases the interpretation of the relative contribution of each respiratory component is different. In the absence of cytochrome inhibitors:

$V_t$ = total oxygen consumption

$V_{alt} = V_t -$ oxygen consumption in the presence of SHAM

$V_{res} = $ oxygen consumption in the presence of both SHAM and cyanide/azide

$V_{cyt} = $ oxygen consumption in the presence of SHAM $- V_{res}$

In the absence of inhibitors of cytochrome oxidase $V_{cyt}$ represents the actual contribution of the cytochrome pathway while $V_{alt}$ represents the actual (engaged) contribution of the alternative pathway. In the presence of cytochrome inhibitors:

$V_t = $ total oxygen consumption

$V_{alt} = $ oxygen consumption in the presence of KCN $- V_{res}$
In the presence of cytochrome oxidase inhibitors, $V_{alt}$ represents the potential capacity of the alternative pathway which may or may not be engaged when the cytochrome pathway is not blocked.

E. Statistical analysis and graphics.

All experiments were repeated two or more times with similar results. Analysis of variance was performed for each experiment (U.B.C. MFAV). Student-Newman-Keul's multiple range test (SNK MRT) and least significant difference (LSD) values were computed (U.B.C. MFAV and Completely Randomized and Randomized Complete Block Analysis of Variance program by D.C. Koller and N.R. Knowles, respectively) for convenient mean separation when the F-value for the main effect of treatment was significant.
RESULTS

A. The induction and release of secondary dormancy


Anaerobiosis induced secondary dormancy in after-ripened seeds of dormant lines (AN-51 and Mont 73, Fig. 2). The optimum duration of anaerobiosis (i.e. days of anaerobiosis for maximum induction of dormancy) varied from 1 to 4 days for AN-51 and from 4 to 10 days for Mont 73; shorter periods were less effective whereas longer periods released secondary dormancy (Fig. 2). The maximum dormancy induced was consistently greater than 85% in line AN-51, but varied substantially for Mont 73. In general, secondary dormancy could be induced more effectively in line AN-51 than in Mont 73.

Pre-imbibition of after-ripened seeds of line AN-51 under aerobic conditions affected their response to subsequent anaerobic treatment. With up to 4 h of pre-imbibition, secondary dormancy could be induced in 85% of the seeds (Fig. 3). Dormancy was induced to a lesser extent with longer (4 to 12 h) pre-imbibition. When seeds were imbibed aerobically for longer than 12 h, visible germination occurred prior to and during anaerobiosis. Anaerobic treatment of these seeds significantly affected their ability to develop into seedlings (>1 cm coleoptile and radicle) and reduced their viability (Fig. 3).

In contrast to the response of dormant lines, seeds of nondormant lines (CS-40, SH-430) developed a short dormancy (2 to 3 weeks; Fig.
Final germination was recorded 30 days after the completion of anaerobiosis. Values are the means of 4 replicates. F-value for interaction of line X time was significant at the p= 0.05 level.
Fig. 3 Induction of secondary dormancy in aerobically pre-imbibed after-ripened seeds of line AN-51.

\[ \Delta \], % germination immediately before anaerobiosis (LSD = 6%); \[ \times \], % germination immediately after anaerobiosis (LSD = 2%); \[ \square \], % germination 24 days after anaerobiosis; \[ \bullet \], % seedling establishment (LSD = 29%). Seeds were imbibed under aerobic conditions for the specified period and then subjected to 3 day anaerobiosis. Seedlings establishment refers to germinated seeds with a coleoptile and radicle >1 cm.
4). Secondarily dormant seeds of dormant lines did not germinate for up to 10 months in an imbibed state (at 25 C, data not shown). After-ripened seeds of these lines, not having received anaerobic treatment, gave germination rates of 100% in less than 3 days when imbibed under aerobic conditions. In contrast, secondarily dormant seeds of nondormant lines showed a rapid increase in germination 1 to 2 weeks after completion of anaerobiosis (Fig. 4). This increase always occurred earlier in line CS-40 than in line SH-430 (Fig. 4) and was not affected by the presence of 3 mM SHAM (Tab. 1). The optimum period of anaerobiosis for both nondormant lines varied between 4 to 10 days (data not shown).

Secondarily dormant seeds of line AN-51 after-ripened rapidly during dry storage; germination increased from zero to > 90% over 22 weeks of dry storage (Fig. 5).


Temperature during anaerobiosis significantly affected the induction of secondary dormancy in lines Mont 73, SH-430 and CS-40; higher temperatures (25 and 30 C) being more effective at inducing dormancy (Tab. 2). In Mont 73 this effect was evident only with short (1 day) anaerobiosis (Tab. 2); with a 3 day anaerobiosis treatment temperature did not affect the induction of dormancy in dormant lines (data not shown).
Fig. 4 The induction of secondary dormancy in genetically nondormant seeds of lines SH-430 and CS-40.

Seeds were subjected to 4 day anaerobiosis. Germination was then monitored under aerobic conditions. Values are the means of 4 replicates. Bars represent the LSD (0.05) at 9 and 13 days after anaerobiosis.
Table 1. Effect of SHAM on the release of secondary dormancy in line SH-430.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage germination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 days</td>
</tr>
<tr>
<td>Water control</td>
<td>20a</td>
</tr>
<tr>
<td>SHAM, 3 mM</td>
<td>23a</td>
</tr>
</tbody>
</table>

Secondary dormancy was induced by a 3-day anaerobiosis treatment; percent germination was recorded at 7, 14 and 21 days thereafter. Each value is the mean of four replicates. Values within columns and rows that are followed by the same letter do not differ significantly at the p= 0.05 level according to the Student Newman Keul's MRT. F-value for difference from control was not significant at the p= 0.05 level.
Fig. 5 After-ripening of secondarily dormant seeds of line AN-51 during dry storage.

After-ripened seeds were subjected to four day anaerobiosis, air-dried and stored at room temperature. Germination was tested at the indicated intervals. Values represent the means of 4 replicates.
Table 2. Effect of anaerobiosis temperature on the induction of secondary dormancy in seeds of nondormant (SH-430, CS-40) and after-ripened seeds of dormant (AN-51, Mont 73) lines.

<table>
<thead>
<tr>
<th>Anaerobiosis temperature (°C)</th>
<th>Experiment I</th>
<th>Experiment II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AN-51</td>
<td>Mont 73</td>
</tr>
<tr>
<td>15</td>
<td>18a</td>
<td>55b</td>
</tr>
<tr>
<td>20</td>
<td>10a</td>
<td>45b</td>
</tr>
<tr>
<td>25</td>
<td>8a</td>
<td>15a</td>
</tr>
<tr>
<td>30</td>
<td>5a</td>
<td>0a</td>
</tr>
</tbody>
</table>

Line \* ns
Temperature \* *
Line X Temperature \* ns

*F-value was significant at the p= 0.05 level, ns - not significant.

Secondary dormancy was induced by 3-day anaerobiosis in the nondormant and 1-day anaerobiosis in the dormant lines; germination was recorded at 6 and 25 days after anaerobiosis in nondormant and dormant lines, respectively. Each value is the mean of four replicates; values within columns and rows that are followed by the same letter do not differ significantly at the p= 0.05 level according to the SNK MRT.
Seed age had a pronounced effect on the induction of secondary dormancy in a nondormant line. Secondary dormancy in SH-430 could be induced only in seeds stored for less than 2 months. Older seeds failed to acquire secondary dormancy under these conditions (Tab. 3). Seeds used in this experiment germinated 100% within 3 days when not subjected to anaerobiosis. On the other hand, seeds of dormant lines were able to acquire secondary dormancy even after 2 years of after-ripening.

3. Stimulation of germination of secondarily dormant seeds.

Ethanol (175 mM), kinetin (1 mM), isopentenyl adenine (1 mM), potassium nitrate (100 mM) and sodium azide (1 mM) stimulated germination (by >85%) in secondarily dormant seeds of line AN-51 (Tab. 4). With the exception of azide, SHAM did not inhibit the promotion of germination by these stimulants (Tab. 4). Germination was increased with SHAM alone and reduced in the presence of azide and SHAM.

4. Determination of respiratory components.

Azide and cyanide were compared for their relative effectiveness as inhibitors of cytochrome-mediated respiration in excised embryos. Maximal inhibition of respiration was greater with cyanide (65%) than with azide (15%) (Fig. 6 and 7). Cyanide was also effective at lower concentrations than azide; maximal inhibition was achieved with 0.5 mM cyanide (Fig 6.), whereas 1.5 mM azide (Fig. 7) was required to elicit
Table 3. Effect of seed age on the induction of secondary dormancy in nondormant line SH-430.

<table>
<thead>
<tr>
<th>Seed age</th>
<th>Percent germination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 days</td>
</tr>
<tr>
<td>5 weeks</td>
<td>0a</td>
</tr>
<tr>
<td>7 weeks</td>
<td>20b</td>
</tr>
<tr>
<td>4 months</td>
<td>100c</td>
</tr>
<tr>
<td>7 months</td>
<td>98c</td>
</tr>
<tr>
<td>14 months</td>
<td>100c</td>
</tr>
</tbody>
</table>

Secondary dormancy was induced by 5-day anaerobiosis. Each value is the mean of four replicates; values within columns followed by the same letter do not differ significantly at the p= 0.05 level according to the SNK MRT. The F-values for seed age and observation time were significant at the p= 0.05 level; the interaction was not significant.
Table 4. Stimulation of germination of secondarily dormant seeds (line AN-51) by chemicals known to break primary dormancy.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percent germination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>- SHAM</td>
</tr>
<tr>
<td></td>
<td>(3 mM)</td>
</tr>
<tr>
<td>Control</td>
<td>13a</td>
</tr>
<tr>
<td>Kinetin (1 mM)</td>
<td>100d</td>
</tr>
<tr>
<td>Isopentenyl adenine (1 mM)</td>
<td>100d</td>
</tr>
<tr>
<td>Sodium azide (1 mM)</td>
<td>98d</td>
</tr>
<tr>
<td>Potassium nitrate (100 mM)</td>
<td>93d</td>
</tr>
<tr>
<td>Ethanol (175 mM)</td>
<td>85d</td>
</tr>
</tbody>
</table>

Germination was recorded 12 days after the onset of treatment. Each value is the mean of four replicates. Values within columns and rows followed by the same letter do not differ significantly at the p=0.05 level according to the SNK MRT. F-values for the main effects of germination stimulants, +/- SHAM and their interaction were significant at the p=0.05 level.
Fig. 6 The effect of cyanide on the respiration of excised embryos (line AN-51).

Embryos were excised from seeds imbibed on water for two hours. KCN was injected directly into the oxygen-uptake vessel after a linear rate of oxygen consumption had been recorded. Values represent the means of 3 replicates.
Fig. 7 The effect of azide on the respiration of excised embryos (line AN-51).

Embryos were excised from seeds imbibed on water for two hours. Azide was injected directly into the oxygen-uptake vessel after a linear rate of oxygen consumption had been recorded. Values represent the means of 3 replicates.
maximum respiratory inhibition. At low concentrations (0.25 – 1.25 mM) azide stimulated embryo respiration by 7 to 12%. Consequently, cyanide was used to inhibit cytochrome-mediated respiration in subsequent experiments involving excised embryos. SHAM alone did not inhibit oxygen uptake of excised embryos or whole seeds (Tab. 5). It did, however, inhibit the respiration of excised embryos (by 35 to 50%) in the presence of cyanide (Tab. 5). SHAM (5 and 10 mM) was not effective at blocking oxygen uptake in the presence of 1 mM cyanide in intact seeds (Tab. 5).

5. Respiratory components of embryos excised from after-ripened, primarily and secondarily dormant seeds.

Relative contributions of CN-sensitive and SHAM-sensitive (alternative) pathways to total respiration of embryos excised from nongerminated seeds (after-ripened, primarily dormant and secondarily dormant) were determined. The induction of secondary dormancy had no effect on the total respiration of embryos excised from secondarily dormant seeds (Tab. 6). Primarily dormant seeds had higher total respiration, almost all of which was cytochrome-mediated (Tab. 6). Cyanide (1 mM) substantially inhibited respiration (by 44 to 85%) in all cases (Tab. 6), SHAM alone had no significant effect on oxygen-uptake regardless of dormancy status (Tab. 6). Oxygen-uptake in the presence of cyanide was greater in secondarily dormant and after-ripened seeds than in primarily dormant seeds (Tab. 6). Residual respiration was similar in all cases (12-16% of total respiration – Tab. 6).
Table 5. Inhibition of seed and embryo respiration by 1 mM cyanide in the presence or absence of SHAM.

<table>
<thead>
<tr>
<th>Oxygen uptake</th>
<th>Oxygen uptake (nmol min(^{-1}) seed(^{-1}))</th>
<th>Seed</th>
<th>Embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>initial +KCN</td>
<td>initial +KCN</td>
<td></td>
</tr>
<tr>
<td>Buffer control</td>
<td>0.53a 0.26b (51)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.32a 0.14b (56)</td>
<td></td>
</tr>
<tr>
<td>5 mM SHAM</td>
<td>0.54a 0.24b (56)</td>
<td>0.28a 0.07c (75)</td>
<td></td>
</tr>
<tr>
<td>10 mM SHAM</td>
<td>0.53a 0.23b (57)</td>
<td>0.30a 0.09c (70)</td>
<td></td>
</tr>
</tbody>
</table>

| SHAM          | ns                                             | ns               |
| KCN           | *                                              | *                |
| SHAM X KCN    | ns                                             | *                |

*F-value significant at the p= 0.05 level, ns - not significant.

<sup>1</sup>Values in brackets represent % inhibition compared to initial oxygen uptake. Seeds were imbibed on water for 24 h. Embryos were excised from seeds imbibed on water for 2 h. Oxygen uptake was determined in 50 mM phosphate buffer (pH 6.9) with or without SHAM. After a linear rate of oxygen uptake had been established 10 uL of concentrated KCN solution was injected directly into the oxygen uptake vessel to achieve 1 mM KCN concentration and subsequent oxygen uptake monitored. Values are the means of three replicates. Means followed by the same letter do not differ significantly at the p= 0.05 level according to the SNK MRT.
Table 6. Effect of respiratory inhibitors on the oxygen uptake of embryos excised from after-ripened, primarily and secondarily dormant seeds of line AN-51.

<table>
<thead>
<tr>
<th>Dormancy status</th>
<th>Buffer only</th>
<th>KCN (V&lt;sub&gt;res&lt;/sub&gt;)</th>
<th>SHAM (1)</th>
<th>KCN + SHAM (2)</th>
<th>V&lt;sub&gt;cyt&lt;/sub&gt; (3)</th>
<th>V&lt;sup&gt;1&lt;/sup&gt; alt (3 - 4)</th>
<th>V&lt;sup&gt;2&lt;/sup&gt; alt (1 - 3)</th>
<th>V&lt;sup&gt;2&lt;/sup&gt; alt (2 - 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>After-ripened</td>
<td>0.18a</td>
<td>0.07c</td>
<td>0.18a</td>
<td>0.02d</td>
<td>0.16</td>
<td>0</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Secondarily</td>
<td>0.18a</td>
<td>0.10c</td>
<td>0.16a</td>
<td>0.03d</td>
<td>0.13</td>
<td>0</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Primarily</td>
<td>0.27b</td>
<td>0.04d</td>
<td>0.28b</td>
<td>0.03d</td>
<td>0.25</td>
<td>0</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Engaged (in the absence of KCN); <sup>2</sup>potential (in the presence of KCN).

Inhibitor concentrations were 1 mM KCN and 5 mM SHAM. Intact seeds were imbibed for 2 h on water prior to embryo excision. Oxygen uptake was determined in 50 mM phosphate buffer with or without SHAM. KCN was injected directly into the oxygen uptake vessel and subsequent oxygen uptake recorded. Values are the means of 3 replicates. Means within columns and rows followed by the same letter do not differ significantly at the p= 0.05 level according to the SNK MRT. F-values for the main effects of dormancy status, inhibitor treatment and their interaction were significant at the p= 0.05 level.
B. Effects of respiratory inhibitors on seed germination

1. The release of primary and secondary dormancy by azide and cyanide

Experiments comparing the release of primary and secondary dormancy by the cytochrome oxidase inhibitors cyanide and azide were performed. Cyanide effectively promoted the release of primary dormancy at concentrations ranging from 0.3 to 3 mM, with 0.5 and 1.0 mM giving the highest germination percentages (Tab. 7). Germination stimulated by 1 and 3 mM cyanide was delayed several days (cf. germination stimulated by 0.5 mM cyanide) (Tab. 7). It was necessary to seal Petri plates containing cyanide with Parafilm to retard loss of HCN gas. No stimulation of germination occurred when cyanide (0.5-10.0 mM) was applied in unsealed dishes (data not shown).

To determine the optimal duration of azide and cyanide treatment for breaking dormancy, inhibitor treatments were given for different durations and germination was monitored. The resulting curve for azide was quadratic (Fig. 8). In this experiment maximum release of dormancy occurred after 4-12 h azide treatment (Fig. 8). Although longer (i.e. 16-24 h) treatments were less effective at releasing dormancy, continuous azide treatment broke dormancy to a similar extent as the treatment for optimum duration (4 to 12 h, Fig. 8) but germination was delayed up to 8 days (Tab. 8).

Cyanide treatments of different duration gave a similar dormancy release pattern (Fig. 9) as found with azide (Fig. 8). Four to 8 hour cyanide treatment resulted in maximum release of dormancy with the effectiveness of treatment declining (although to a lesser extent than with azide) with longer pulse durations (Fig. 9). Similarly maximum
Table 7. The effect of cyanide on percentage and rate of germination in primarily dormant seeds of line AN-51.

<table>
<thead>
<tr>
<th>Cyanide concentration (mM)</th>
<th>Percent germination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 day</td>
</tr>
<tr>
<td>0</td>
<td>0a</td>
</tr>
<tr>
<td>0.10</td>
<td>0a</td>
</tr>
<tr>
<td>0.30</td>
<td>5a</td>
</tr>
<tr>
<td>0.50</td>
<td>48b</td>
</tr>
<tr>
<td>1.0</td>
<td>8a</td>
</tr>
<tr>
<td>3.0</td>
<td>0a</td>
</tr>
</tbody>
</table>

Seeds were treated with the indicated concentrations of cyanide; germination was recorded 2, 3 and 5 days after the onset of treatment. Values represent the means of four replicates. Means within columns followed by the same letter do not differ at the p = 0.05 level according to the SNK MRT. F-values for the main effects of cyanide treatment, observation time and their interaction were significant at the p = 0.05 level.
Fig. 8 Effect of duration of azide treatment on the release of primary dormancy in seeds of line AN-51.

Seeds were treated with 1 mM azide for the indicated duration and then transferred to distilled water for the remainder of the experiment. Germination was recorded 14 days after azide treatment. Values represent the means of 4 replicates.
Table 8. The effect of duration of azide (1 mM) treatment on the percentage and the rate of germination.

<table>
<thead>
<tr>
<th>Treatment duration (h)</th>
<th>Percentage germination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day</td>
</tr>
<tr>
<td>0</td>
<td>8a</td>
</tr>
<tr>
<td>4</td>
<td>63b</td>
</tr>
<tr>
<td>8</td>
<td>43c</td>
</tr>
<tr>
<td>16</td>
<td>0a</td>
</tr>
<tr>
<td>Continuous</td>
<td>0a</td>
</tr>
</tbody>
</table>

Primarily dormant seeds of line AN-51 were treated with azide for the specified period, transferred to distilled water and germination recorded at the indicated intervals. Values are the mean of four replicates. Means within columns followed by the same letter do not differ significantly at the \( p = 0.05 \) level according to the SNK MRT. F-values for the main effects of azide treatment, observation time and their interaction were significant at the \( p = 0.05 \) level.
Fig. 9 Effect of duration of cyanide treatment on the release of primary dormancy in seeds of line AN-51.

Seeds (4 replicates) were treated with 1 mM cyanide for the indicated duration and then transferred to distilled water for the remainder of the experiment. Germination was recorded 14 days after cyanide treatment. Values represent the means of 4 replicates.
release of dormancy occurred with continuous 1 mM cyanide treatment (Tab. 7) but, unlike azide, germination was much quicker (Tab. 8).

Although 1 mM azide effectively released both primary and secondary dormancy in seeds after-ripened for 3 months in one experiment (Fig. 8), it was ineffective at releasing seed dormancy in 2 to 5 month old seeds of AN-51 when applied continuously or as a pulse treatment in a second experiment (Tab. 9). On the other hand, 0.5 mM cyanide stimulated seed germination regardless of the duration of after-ripening although to different extents (Tab. 9).

2. The effect of SHAM on the release of dormancy by cyanide and azide.

SHAM (10 mM) applied simultaneously with 1 mM azide, completely inhibited germination of secondarily dormant seeds (Tab. 10). In order for SHAM to prevent the release of secondary dormancy by a 6 h azide pulse treatment it was necessary for SHAM to be present continually (i.e. during and after the 6 h pulse; Tab. 10). SHAM applied subsequent to the azide pulse had little effect on the release of dormancy; likewise, when present only during the 6 h azide pulse, SHAM failed to inhibit azide-induced germination. SHAM applied simultaneously with cyanide had no effect on the stimulation of germination by the latter compound (Tab. 10).
Table 9. Effect of after-ripening on the release of dormancy by azide and cyanide in line AN-51.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Months after-ripening</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Control</td>
<td>5a*</td>
</tr>
<tr>
<td>Cyanide</td>
<td>70b</td>
</tr>
<tr>
<td>Azide</td>
<td>0a</td>
</tr>
<tr>
<td>Azide - 6 h pulse</td>
<td>0a</td>
</tr>
</tbody>
</table>

*Percent germination.

Seeds of the indicated ages were treated with either continuous 0.5 mM KCN, 1.0 mM azide or a 6 h pulse of 1.0 mM azide followed by water imbibition. Final germination percentages were recorded 14 days after treatment. Values are the mean of four replicates. Means within columns and rows followed by the same letter do not differ significantly at the p= 0.05 level according to the SNK MRT. F-values for the main effects of seed age, inhibitor treatment and their interaction were significant at the p= 0.05 level.
Table 10. The effect of SHAM on the release of secondary dormancy by azide and cyanide.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percent germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7a</td>
</tr>
<tr>
<td>SHAM</td>
<td>7a</td>
</tr>
<tr>
<td>Azide</td>
<td>87b</td>
</tr>
<tr>
<td>Cyanide</td>
<td>77b</td>
</tr>
<tr>
<td>Azide + SHAM</td>
<td>0a</td>
</tr>
<tr>
<td>Cyanide + SHAM</td>
<td>83b</td>
</tr>
<tr>
<td>6 h Azide --&gt; water*</td>
<td>97b</td>
</tr>
<tr>
<td>6 h Azide --&gt; SHAM</td>
<td>70b</td>
</tr>
<tr>
<td>6 h Azide + SHAM --&gt; water</td>
<td>90b</td>
</tr>
<tr>
<td>6 h Azide + SHAM --&gt; SHAM</td>
<td>15a</td>
</tr>
</tbody>
</table>

*Six h inhibitor treatment was followed by transfer (--> to water or SHAM for the remainder of the experiment. Germination was recorded 14 days after the onset of treatment. Inhibitor concentrations used were 1 mM azide, 0.5 mM cyanide and 10 mM SHAM. Values are the means of 4 replicates. Means followed by the same letter do not differ significantly at the p= 0.05 level according to the SNK MRT.
C. Effect of respiratory inhibitors on seed germination and oxygen uptake.

1. Stimulation of seed germination and respiration by azide and cyanide. Cyanide stimulated seed respiration to an extent similar to azide over the same treatment period (Tab. 11). Cyanide concentrations ranging from 0.05 mM to 1.0 mM gave comparable release of dormancy (Fig. 10). Oxygen-uptake increased linearly with increasing log cyanide concentration with maximum stimulation occurring at 1 mM cyanide. Respiration dropped sharply at 3 mM cyanide and germination was completely inhibited at this concentration (Fig. 10).

   Short cyanide pulses (2 and 4 h) stimulated respiration to a similar extent (33 and 45% over the control, respectively - Fig. 11). Germination was stimulated by both treatments and did not differ according to the SNK MRT. Shorter pulse treatments (30 and 60 min) stimulated respiration by 20 to 25% but did not release dormancy (Tab. 12).

2. The nature of azide and cyanide-induced respiration.

   Azide and cyanide stimulated oxygen uptake to a similar extent (77-79%) when applied continuously (Tab. 11). SHAM (10 mM) applied concurrently not only prevented the stimulation of respiration by azide but also reduced it far below that of the control (by 68%); SHAM had no effect on the stimulation of respiration by cyanide (Tab. 11). Removing seeds from azide and cyanide after 24 h and placing them on water for a subsequent 14 h resulted in lower oxygen uptake than the seeds receiving continuous cyanide or azide (i.e. 50-58% above the
Fig. 10. Effect of cyanide on respiration and germination of primarily dormant seeds of line AN-51.

Oxygen uptake of intact seeds was recorded 24 h following KCN treatment. Control seeds did not germinate and had a respiratory rate of 0.60 nmol min$^{-1}$ seed$^{-1}$. Germination was recorded 5 days after the onset of treatment. Values represent the means of 3 replicates.
Fig. 11 Effect of cyanide pulse treatments on the respiration and germination of primarily dormant seeds of line AN-51.

Seeds were treated with 1 mM KCN for 2 or 4 h and then transferred to water; oxygen-uptake was then recorded at the specified intervals. Germination (recorded 5 days after the initial treatment) was: control, 0%; 2 h pulse, 66%; 4 h pulse, 87%. Values represent the means of 3 replicates.
Table 11. Effect of SHAM on the induction of respiration by 24 h azide or cyanide treatment in primarily dormant seeds of line AN-51.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oxygen uptake (nmol min(^{-1}) seed(^{-1}))</th>
<th>Percent of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.56a</td>
<td>100</td>
</tr>
<tr>
<td>Cyanide</td>
<td>1.00b</td>
<td>179</td>
</tr>
<tr>
<td>Azide</td>
<td>1.01b</td>
<td>180</td>
</tr>
<tr>
<td>SHAM</td>
<td>0.60a</td>
<td>107</td>
</tr>
<tr>
<td>Cyanide + SHAM</td>
<td>1.09b</td>
<td>195</td>
</tr>
<tr>
<td>Azide + SHAM</td>
<td>0.18c</td>
<td>32</td>
</tr>
</tbody>
</table>

Inhibitor concentrations used were 1 mM azide and cyanide and 10 mM SHAM. Values are the means of three replicates. Means followed by the same letter do not differ significantly at the p = 0.05 level according to the SNK MRT.
Table 12. The effect of short cyanide pulse treatments on respiration and germination in primarily dormant seeds of line AN-51.

<table>
<thead>
<tr>
<th>Pulse duration (min)</th>
<th>Oxygen uptake (nmol min⁻¹ seed⁻¹)</th>
<th>% Over</th>
<th>% Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.67a</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>0.84b</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>60</td>
<td>0.80b</td>
<td>19</td>
<td>0</td>
</tr>
</tbody>
</table>

Seeds were treated with 1 mM KCN for 30 or 60 minutes and then transferred to water for the duration of the experiment. Oxygen consumption was recorded 24 h after the onset of the pulse. Values represent the mean of 2 replicates; means followed by the same letter do not differ at the p= 0.05 level according to the SNK MRT.
Table 13. The nature of azide- and cyanide-induced respiration in primarily dormant seed of line AN-51.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oxygen-uptake (nmol seed$^{-1}$ min$^{-1}$)</th>
<th>Percent increase over control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water --&gt; water</td>
<td>0.69a</td>
<td>--</td>
</tr>
<tr>
<td>Cyanide --&gt; water</td>
<td>1.04b</td>
<td>51</td>
</tr>
<tr>
<td>Cyanide --&gt; cyanide</td>
<td>1.30de</td>
<td>88</td>
</tr>
<tr>
<td>Cyanide --&gt; SHAM</td>
<td>1.09bc</td>
<td>58</td>
</tr>
<tr>
<td>Cyanide --&gt; cyanide + SHAM</td>
<td>1.24de</td>
<td>80</td>
</tr>
<tr>
<td>Cyanide --&gt; azide + SHAM</td>
<td>1.21cd</td>
<td>75</td>
</tr>
<tr>
<td>Cyanide --&gt; azide</td>
<td>1.38e</td>
<td>100</td>
</tr>
<tr>
<td>Azide --&gt; water</td>
<td>1.11bc</td>
<td>61</td>
</tr>
<tr>
<td>Azide --&gt; cyanide</td>
<td>1.37e</td>
<td>99</td>
</tr>
<tr>
<td>Azide --&gt; azide</td>
<td>1.36e</td>
<td>97</td>
</tr>
<tr>
<td>Azide --&gt; SHAM</td>
<td>1.33de</td>
<td>93</td>
</tr>
<tr>
<td>Azide --&gt; cyanide + SHAM</td>
<td>1.37e</td>
<td>99</td>
</tr>
<tr>
<td>Azide --&gt; azide + SHAM</td>
<td>1.01b</td>
<td>46</td>
</tr>
</tbody>
</table>

Seeds were incubated in water, azide or cyanide for 24 h and then transferred (--> ) to the specified solutions for 14 h and oxygen uptake subsequently recorded. Inhibitor concentrations used were 1 mM azide and cyanide and 10 mM SHAM. Values are the means of two replicates. Means followed by the same letter do not differ significantly at the p= 0.05 level according to the SNK MRT.
control); renewing azide and cyanide solutions after the initial 24 h treatment resulted in increased respiration in the following 14 h period (Tab. 13). SHAM (10 mM) applied after a 24 h cyanide or azide treatment did not inhibit respiration either alone or when applied in combination with azide or cyanide. SHAM did, however, prevent further stimulation of respiration by azide when applied in combination with 1 mM azide for the 14 h period subsequent to the initial 24 h azide treatment (Tab. 13).

The respiration of dormant seed treated with high concentrations of azide (5–20 mM) for 5 h (preliminary experiments showed 5 h to be adequate for maximum inhibition at these azide concentrations) was measured (Tab. 14). Azide reduced oxygen uptake in intact seeds by approximately 40% at all concentrations. The respiration of seeds treated with azide plus 1 or 5 mM SHAM did not differ significantly from seeds treated with 5 mM azide alone after a 5 h incubation (Tab. 15). Applied concurrently with 5 mM azide, 10 and 20 mM SHAM inhibited respiration below that of seeds receiving 5 mM azide alone or with 1 or 5 mM SHAM (Tab. 15).

3. Partitioning of azide and cyanide-induced respiration.

The respiratory components of embryos excised from non-germinated seeds treated with either 1 mM cyanide or azide for 24 h were determined. SHAM (5 mM) alone did not significantly inhibit the respiration of embryos excised from water, azide or cyanide imbibed seeds (Tab. 16). Respiration of the control embryos was inhibited 45% by 1 mM cyanide but the respiration of embryos excised from azide and
Table 14. Dose-response for the inhibition of seed respiration by azide.

<table>
<thead>
<tr>
<th>Azide concentration (mM)</th>
<th>Oxygen uptake (nmol seed⁻¹ min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.66a</td>
</tr>
<tr>
<td>5</td>
<td>0.41b</td>
</tr>
<tr>
<td>10</td>
<td>0.42b</td>
</tr>
<tr>
<td>15</td>
<td>0.38b</td>
</tr>
<tr>
<td>20</td>
<td>0.41b</td>
</tr>
</tbody>
</table>

Seeds were imbibed on an azide solution for 5 h after which oxygen uptake was recorded. Values represent the mean of 3 replicates. Means followed by the same letter do not differ significantly at the p= 0.05 level according to the SNK MRT. F-value for comparison of all azide concentrations vs. control was significant at the p= 0.05 level.
Table 15. Dose-response for the effect of SHAM on the inhibition of seed respiration by 5 mM azide.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oxygen uptake (nmol seed(^{-1}) min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (water)</td>
<td>0.82a</td>
</tr>
<tr>
<td>Azide (5 mM)</td>
<td>0.43b</td>
</tr>
<tr>
<td>&quot; + SHAM (1 mM)</td>
<td>0.41b</td>
</tr>
<tr>
<td>&quot; + SHAM (5 mM)</td>
<td>0.46b</td>
</tr>
<tr>
<td>&quot; + SHAM (10 mM)</td>
<td>0.34c</td>
</tr>
<tr>
<td>&quot; + SHAM (20 mM)</td>
<td>0.33c</td>
</tr>
</tbody>
</table>

Seeds were imbibed for 5 h on 5 mM azide with or without the specified concentrations of SHAM and oxygen uptake was recorded. Values are the mean of 3 replicates. Means followed by the same letter do not differ significantly at the p= 0.05 level according to SNK MRT.
Table 16. The effect of cyanide and azide pretreatment of primarily dormant intact seeds on the response of excised embryos to respiratory inhibitors (line AN-51).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>buffer</th>
<th>KCN**</th>
<th>SHAM</th>
<th>KCN + SHAM</th>
<th>( V_{\text{cyt}} )</th>
<th>( V_{\text{alt}}^1 )</th>
<th>( V_{\text{alt}}^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1)</td>
<td>(2)</td>
<td>(3)</td>
<td>(4)</td>
<td>(3 - 4)</td>
<td>(1 - 3)</td>
<td>(2 - 4)</td>
</tr>
<tr>
<td>Control</td>
<td>0.29a</td>
<td>0.16b</td>
<td>0.28a</td>
<td>0.06c</td>
<td>0.22</td>
<td>0.01</td>
<td>0.10</td>
</tr>
<tr>
<td>Azide</td>
<td>0.69f</td>
<td>0.61ef</td>
<td>0.60ef</td>
<td>0.41d</td>
<td>0.19</td>
<td>0.09</td>
<td>0.20</td>
</tr>
<tr>
<td>Cyanide</td>
<td>0.66ef</td>
<td>0.60ef</td>
<td>0.65ef</td>
<td>0.56e</td>
<td>0.09</td>
<td>0.01</td>
<td>0.04</td>
</tr>
</tbody>
</table>

1Engaged (in the absence of KCN); 2potential (in the presence of KCN).

*Intact seeds were imbibed for 24 h on water, or 1 mM azide or cyanide. Embryos were then excised and their oxygen uptake recorded in 50 mM phosphate buffer (pH 6.9) with or without inhibitors (1.0 mM KCN and/or 5 mM SHAM) as specified (**). Values are the means of 3 replicates. Means within columns and rows followed by the same letter do not differ significantly at the p= 0.05 level according to the SNK MRT. F-values for the main effects of pretreatment, respiratory inhibitors applied during oxygen uptake and their interaction were significant at the p= 0.05 level.
cyanide treated seeds was not strongly inhibited (Tab. 16). In the presence of SHAM, cyanide inhibited respiration in the control (79%) far more than in embryos of either cyanide or azide treated seeds (Tab. 16).

D. Effect of pH on the action of respiratory inhibitors

1. Respiration studies

Substantial pH changes occur when SHAM, cyanide, and azide are dissolved in distilled water (Tab. 17). Experiments were performed to determine the effect of pH on the action of respiratory inhibitors. The respiration of dormant seeds imbibed on water or citrate-phosphate buffer (pH 5 and 7) did not differ significantly (Tab. 17). Similarly SHAM alone did not affect respiration regardless of pH or buffering. Cyanide, with or without SHAM stimulated oxygen uptake in the buffered systems and in water to the same extent (Tab. 17). On the other hand, pH had a striking effect on the action of azide with or without SHAM. In water and in buffer (pH 7), 1 mM azide stimulated seed respiration, however at pH 5 respiration was strongly inhibited (by 85%). At pH 7, azide + SHAM not only failed to inhibit respiration (cf. the control) but actually stimulated oxygen consumption by over 20% (Tab. 17). On the other hand, at pH 5 azide + SHAM inhibited respiration by 85%. In dose-response studies, seed respiration was stimulated by azide (pH 7) at concentrations ranging from 0.065 to 2.0 mM (Fig. 12). However, respiration was sharply inhibited by azide concentrations over 0.125 and 0.5 mM at pH 5 and 6, respectively (Fig. 12). The effect of cyanide on seed respiration was not pH sensitive (Fig. 13).
Table 17. The effect of pH on the action of respiratory inhibitors as they effect oxygen uptake of primarily dormant seeds of line AN-51.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Solution pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>non-buffered</td>
</tr>
<tr>
<td>Control</td>
<td>0.81b¹ [5.2]²</td>
</tr>
<tr>
<td>Azide</td>
<td>1.43c [6.2] (77)³</td>
</tr>
<tr>
<td>Azide + SHAM</td>
<td>0.27a [5.2] (-67)</td>
</tr>
<tr>
<td>Cyanide</td>
<td>1.37c [9.6] (69)</td>
</tr>
<tr>
<td>Cyanide + SHAM</td>
<td>1.38c [6.0] (70)</td>
</tr>
<tr>
<td>SHAM</td>
<td>0.78b [3.9] (-4)</td>
</tr>
</tbody>
</table>

¹Oxygen uptake (nmol seed⁻¹ min⁻¹).
²Values in brackets represent the pH of inhibitor solutions dissolved in distilled water.
³Values in parentheses represent % inhibition/stimulation compared to the control.

Seeds were treated with respiratory inhibitors (1 mM azide and cyanide, and 10 mM SHAM) dissolved either in distilled water or citrate phosphate buffer (pH 5 or 7) for 24 h before recording oxygen consumption. Values represent the means of 2 replicates; means followed by the same letter do not differ significantly at the p= 0.05 level according to the SNK MRT. F-values for the main effects of buffering, inhibitor treatment and their interaction were significant at the p= 0.05 level.
The effect of pH on azide-induced respiration in primarily dormant seeds of line AN-51.

Oxygen uptake of seeds treated with azide (dissolved in citrate-phosphate buffer) was measured 24 h after the onset of treatment. The value of the controls (indicated by the arrow) at pH 5, 6 and 7 ($\bar{x} = 0.71$ nmol min$^{-1}$ seed$^{-1}$) did not differ significantly. Values represent the means of 2 replicates. F-value for interaction of pH X azide concentration was significant at the p= 0.05 level.
The effect of 10 mM SHAM and pH adjustment (citrate-phosphate buffer) on the stimulation of respiration by a range of azide concentrations is shown in Fig. 14. Respiratory stimulation by azide increased with increasing azide concentration above 0.1 mM. Simultaneous presence of SHAM with azide (> 0.5 mM) resulted in severe respiratory inhibition. However, at low azide concentrations (0.1 – 0.25 mM) in unbuffered medium, SHAM not only failed to inhibit azide-induced respiration but actually enhanced it compared to the control (Fig. 14). When the pH of azide solutions (of different concentrations) was adjusted to the pH of 10 mM SHAM plus corresponding azide concentrations, the oxygen uptake response curve was very similar to the azide dose-response curve in the presence of 10 mM SHAM (Fig. 14). SHAM (10 mM) had little effect on the stimulation of respiration by cyanide regardless of cyanide concentration (Fig. 15).

2. Germination studies

Azide (1 mM) stimulated the release of secondary dormancy when the solution was unbuffered or buffered at pH 6, 7 or 8 (Tab. 18). Stimulation of germination was maximum at pH 6 and 8. At pH 5 the release of dormancy by azide was completely inhibited. On the other hand, cyanide (1 mM) released seed dormancy regardless of medium pH (Tab. 19). Germination was maximum when cyanide was applied in water or buffered at pH 5 (85 and 90%, respectively). When applied at pH 6, 7 or 8, germination was stimulated to a slightly lesser extent (60 – 68%, Tab. 19).
Fig. 13 The effect of pH on cyanide-induced respiration in primarily dormant seeds of line AN-51.

Oxygen uptake of seeds treated with KCN (dissolved in citrate-phosphate buffer) was measured 24 h after the onset of treatment. The value of the controls (indicated by the arrow) at pH 5, 6 and 7 ($\bar{x} = 0.63 \text{ nmol min}^{-1} \text{ seed}^{-1}$) did not differ significantly. Values represent the means of 2 replicates. pH X KCN concentration interaction was not significant at the p= 0.05 level.
Seeds were treated with the specified concentration of azide dissolved in either: △, distilled water, ×, in distilled water with 10 mM SHAM, or □, in citrate-phosphate buffer, the pH of which equalled the pH of the azide + SHAM solution (in distilled water) at any azide concentration. Oxygen-uptake was recorded 24 h after the onset of treatment. Values represent the means of 2 replicates.
Fig. 15 The effect of SHAM on cyanide-induced respiration in dormant seeds of line AN-51.

Oxygen uptake of seeds treated with KCN (with or without 10 mM SHAM) was recorded 24 h after the onset of treatment. Values represent the means of 2 replicates. Interaction of KCN concentration X +/- SHAM was not significant at the p=0.05 level.
Table 18. The effect of pH on the release of secondary dormancy in seeds of line AN-51 by azide.

<table>
<thead>
<tr>
<th>Solution pH</th>
<th>Percent germination</th>
<th>1 mM azide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td></td>
</tr>
<tr>
<td>Unbuffered</td>
<td>5a</td>
<td>80b</td>
</tr>
<tr>
<td>5</td>
<td>5a</td>
<td>5a</td>
</tr>
<tr>
<td>6</td>
<td>5a</td>
<td>93c</td>
</tr>
<tr>
<td>7</td>
<td>5a</td>
<td>78b</td>
</tr>
<tr>
<td>8</td>
<td>15a</td>
<td>100c</td>
</tr>
</tbody>
</table>

Seeds were imbibed in either 50 mM phosphate (pH 6 to 8) or citrate phosphate buffer (pH 5) or water with or without 1 mM azide at the specified pH. Germination was recorded 14 days after the onset of treatment. Values represent the means of 4 replicates. Means within columns and rows followed by the same letter do not differ significantly at the p= 0.05 level according to the SNK MRT. F-values for the main effects of pH, +/- azide and their interaction were significant at the p= 0.05 level.
Table 19. The effect of pH on the release of secondary dormancy in seeds of line AN-51 by cyanide.

<table>
<thead>
<tr>
<th>Solution pH</th>
<th>Percent germination</th>
<th>1 mM cyanide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td></td>
</tr>
<tr>
<td>Unbuffered</td>
<td>0a</td>
<td>85b</td>
</tr>
<tr>
<td>5</td>
<td>0a</td>
<td>90b</td>
</tr>
<tr>
<td>6</td>
<td>0a</td>
<td>68c</td>
</tr>
<tr>
<td>7</td>
<td>0a</td>
<td>65c</td>
</tr>
<tr>
<td>8</td>
<td>0a</td>
<td>60c</td>
</tr>
</tbody>
</table>

Seeds were imbibed on 50 mM phosphate (pH 8) or citrate phosphate buffer (pH 5 to 7) or water with or without 1 mM KCN at the specified pH. Germination was recorded 7 days after the onset of treatment. Values represent the means of 4 replicates. Means within columns and rows followed by the same letter do not differ significantly at the p=0.05 level according to the SNK MRT. F-values for the main effects of pH, cyanide treatment and their interaction were significant at the p=0.05 level.
DISCUSSION

A. The induction and release of secondary dormancy.

Research on wild oats seed dormancy has been confused by the use of seeds collected from heterogenous field populations of wild oats that vary in their dormancy behavior due to both genetic (Naylor and Jana, 1976) and environmental (Naylor and Fedec, 1978; Sawhney and Naylor, 1979, 1980) factors. Observations on field populations are often not applicable to individual biotypes and vise versa. The results presented here add a new dimension to this problem by showing that genetically pure lines differ with regard to optimal conditions for the induction of secondary dormancy and the degree and duration of the dormancy induced. Hay and Cumming (1959), working with seeds collected from field populations, suggested the possibility of obtaining a constant supply of uniformly dormant seeds by inducing secondary dormancy in after-ripened seeds. In light of the variation between pure lines in the induction and expression of secondary dormancy reported in this thesis, induce dormancy in a genetically diverse sample of seeds cannot be considered an adequate method for obtaining homogenous research material.

Variability in the duration of primary (innate) dormancy has been considered a major factor in the survival of wild oats. This variability leads to the maintenance of a large seed bank in the soil rendering the weed less susceptible to control methods aimed at the seedling stage (e.g. cultivation, herbicides).
Variability in secondary dormancy behavior provides yet another mechanism in the overall survival strategy of wild oats. After-ripened seeds of dormant biotypes can acquire secondary dormancy under unfavourable conditions and distribute germination over time due to differences in the duration of acquired dormancy. The significance of secondary dormancy in the survival of genetically nondormant lines is obvious. To survive the harsh winters of the Canadian Prairies, these lines must have a mechanism, like secondary dormancy, whereby germination can be delayed until winter temperatures are low enough to prevent germination. Although in this study only a short-term dormancy was induced in nondormant lines (Fig. 4), it is possible that under field conditions a combination of factors (viz. temperature, carbon dioxide, anaerobiosis, presence of hulls, etc.) could induce a longer-term dormancy. High temperatures are reported to promote the induction of secondary dormancy in curled dock (Totterdell and Roberts, 1979), lettuce (Vidaver and Hsiao, 1975) and cocklebur (Esashi et al., 1978). In the present study greater induction of secondary dormancy was found as the temperature during anaerobiosis increased (Tab. 2). This is in contrast to the findings of Hay (1962). This contradiction may be explained by the difference in the seed used and by the presence of hulls in the earlier study.

The fact that secondary dormancy could be induced in up to 85% of AN-51 seeds following 4 h of aerobic imbibition (Fig. 3) suggests that biochemical changes during this period do not irreversibly commit these seeds to germination.
Several chemically unrelated compounds are known to break primary dormancy in wild oats. These include azide (Fay and Gorecki, 1978; Upadhyaya et al., 1982a), ethanol (Adkin et al., 1984a) nitrate and nitrite ions (Adkins et al., 1984b; Johnson, 1935) and gibberellins (Simpson, 1978). Cytokinins (isopentenyl adenine, kinetin) have been shown to stimulate germination of primarily dormant seeds in red rice (Cohn and Butera, 1982) and wild oats (unpublished results, M.K. Upadhyaya). Collectively, these chemicals provide a valuable tool for the comparison of the physiological characteristics of primary and secondary dormancies. It was found here that azide, nitrate, ethanol, isopentenyl adenine and kinetin stimulated the germination of secondarily dormant seeds (Tab. 4). The similarity in response of primarily and secondarily dormant seeds to such diverse chemicals suggests that the two types of dormancies in wild oats are similar at least in part in their regulation. The loss of secondary dormancy through after-ripening (as occurs with primary dormancy) supports the notion that secondary and primary dormancies are different manifestations of the same physiological state.

B. Alternative respiration and the regulation of secondary dormancy.

In order to test the hypothesis that the induction of secondary dormancy is accompanied by a decline in the activity of alternative respiration the relative contribution of CN-sensitive and insensitive pathways in embryos excised from seeds before and after the induction of secondary dormancy was examined. Respiratory components were
determined for excised embryos rather than intact seed for the following reasons. Firstly, in order to determine respiratory components instantaneously, inhibitors injected into the oxygen-uptake vessel must elicit an immediate inhibition of respiration. Although cyanide alone gave rapid inhibition of respiration of intact seeds (Tab. 5), the lack of additional inhibition in the presence of SHAM indicates that SHAM does not effectively block the alternative pathway of intact seeds compared to excised embryos presumably because of the permeability barrier presented by the seed coat. This would make distinguishing residual respiration from incompletely inhibited alternative respiration difficult. Secondly, the response of excised embryos and intact seeds to respiratory inhibitors is qualitatively the same (Upadhyaya et al., 1983); presumably the partitioning of embryo respiration can be extrapolated to be indicative of its partitioning in intact seeds. This approach is commonly used and accepted in studies involving seed respiration (Leopold and Musgrave, 1980; Miller et al., 1983). Finally, in the event that excision does cause an artifactual change in the respiratory components of embryo tissue, this change would likely be uniform across treatments, thus enabling treatment effects to be distinguished from the effects of mechanical injury.

Based on their findings that several treatments releasing secondary dormancy resulted in a concomitant increase in the participation of the alternative pathway, Esashi et al. (1981) have proposed that secondary dormancy in cocklebur seeds is due to their
inability to perform alternative respiration. In the current study it was found that with the exception of azide treatment, SHAM did not inhibit the stimulation of germination by a diverse group of chemicals (Tab. 4). Thus, as with primary dormancy, a SHAM-sensitive process (presumably alternative respiration) is not a prerequisite for the promotion of germination of secondarily dormant wild oat seeds by these chemicals.

In wild oats, the alternative pathway did not contribute to the respiration of excised embryos regardless of dormancy status (Tab. 6). The after-ripened and secondary dormant embryos did however, have the capacity to divert electrons through the alternative pathway when the cytochrome pathway was blocked with cyanide. In all cases the maximum flux of the alternative pathway was much less than the maximum flux through the cytochrome pathway (Tab. 6). Clearly, these results do not support the hypothesis that alternative respiration (or the potential to perform alternative respiration) is related to dormancy status in wild oats seeds.

The physiological significance of the elevated contribution of the alternative pathway in cocklebur seeds during the release of dormancy must be questioned. Rather than determining the actual participation of the alternative pathway (i.e. indicated by respiratory inhibition in the presence of SHAM alone), Esashi et al. (1982, 1983) measured its potential capacity (i.e. respiration in the presence of cyanide less residual respiration). In no case did the authors show that any dormancy-breaking treatment actually activated the alternative pathway
while the cytochrome pathway was still functioning. Given the failure of SHAM to inhibit: i) the spontaneous release of secondary dormancy in nondormant lines, ii) the release of secondary dormancy by several chemicals and iii) the respiration of embryos excised from both after-ripened and secondarily dormant seed, it is unlikely that — with the possible exception of azide-induced release of dormancy — alternative respiration has any physiological significance in the regulation of secondary dormancy in wild oats.

C. The action of azide and cyanide on the release of seed dormancy

Although cyanide is known to release seed dormancy in several species (Hendricks and Taylorson, 1972) its effect on germination and respiration has not been examined in wild oats. Cyanide and azide inhibit cytochrome-mediated respiration at the same site — cytochrome oxidase (Ikuma and Bonner, 1967). The results presented here suggest that the two inhibitors also have the same action in stimulating germination and respiration in wild oat seeds — presumably via their common action on cytochrome oxidase.

Although generally speaking cyanide and azide stimulated germination in the same concentration range and by similar treatment durations, some differences between the two compounds were apparent. In one experiment, cyanide was more effective at releasing dormancy in seeds with little after-ripening (i.e. 2 months, Tab. 9); azide (1 mM, either as a continuous treatment or as a 6 h pulse — Tab. 9) was completely ineffective at releasing dormancy in 2 to 5 month old seeds.
In a separate experiment, azide (1 mM) released primary dormancy in 3 month old seeds (Fig. 8). The inconsistent release of primary dormancy by azide may be explained by different rates of after-ripening observed in separate harvests of wild oat seed. Variation in after-ripening rate may arise through differing storage conditions (i.e. changes in temperature and relative humidity). Consequently the physiological age of the seed (in relation to dormancy status) may not be the same in all seeds of the same chronological age. Adkins et al. (1984 a,b) have proposed that two distinct germination blocks exist in dormant wild oat seeds, one which is insensitive to azide and is overcome during after-ripening and a second block which can be released by azide (i.e. in partially after-ripened seeds). If cyanide and azide release dormancy by the same mechanism, the fact that cyanide could stimulate germination regardless of the duration of after-ripening contradicts this hypothesis. Accordingly, failure of azide to induce germination in freshly harvested seeds may not be related to its mode of action per se. It is possible that in order for azide to release dormancy in freshly harvested seeds it would have to be applied at concentrations which would completely inhibit subsequent germination. Rather than two distinct blocks characterizing wild oats seed dormancy, it seems more likely that the degree of dormancy follows a continuum that declines with after-ripening.

Germination rate in response to cyanide and azide treatments also differed. Although continuous cyanide and azide treatments gave
comparable release of dormancy, the latter caused germination to be delayed 5-10 days (Tab. 8) cf. 48-72 h with cyanide treatment (Tab. 7). Upadhyaya et al. (1983) found that 1 mM azide had two distinct effects on wild oat seeds: 1. the release of dormancy and 2. the inhibition of germination. Azide (1 mM) was found to delay the germination of nondormant seeds for a period similar to the lag found in the stimulation of germination by azide in dormant seeds (Upadhyaya et al., 1983). The effectiveness of short azide pulses at releasing dormancy without the resultant inhibition of germination supports this idea of two distinct effects of azide. The release of dormancy by azide probably occurs in the early imbibitional stages; the presence of azide after this time is inhibitory to germination (Tab. 8).

Cyanide (0.5 mM) however was not as toxic to germination; at higher concentrations (i.e. 1.0 mM) germination was somewhat delayed (by 3 days, Tab. 7). Although treatment plates were wrapped with Parafilm, the lesser toxicity of cyanide may be due to the volatilization and gradual escape of HCN from the plates (Yu et al., 1981), resulting in sub-toxic levels of this inhibitor.

D. The stimulation of seed respiration by azide and cyanide.

Cyanide stimulated seed respiration to an extent similar to that of azide under identical conditions (24 h treatment at 1 mM) (Tab. 11). Upadhyaya et al. (1983) found that azide (1 mM continuous treatment) stimulated respiration far in advance of germination. However since azide also inhibits germination following the release of
dormancy, it is not possible to determine if respiration was enhanced prior to the actual release of dormancy \textit{per se}.

Because dormancy can be broken by cyanide without an accompanying inhibition of germination, the onset of respiratory stimulation by this compound was examined. To determine if the release of dormancy and the stimulation of respiration by cyanide could be dissociated, two experiments were performed. Figure 10 shows the cyanide dose-response of seed germination and respiration. The release of dormancy (in this experiment) was always accompanied by an increase in seed respiration (measured well in advance of any visible signs of germination). The degree of respiratory stimulation did not however, relate quantitatively to the release of dormancy. Although oxygen-uptake was maximum at 1.0 mM cyanide, release of dormancy varied little between 0.05 and 1.0 mM cyanide. In a second experiment (Fig. 11) seed respiration and germination in response to short cyanide pulses were measured. Both 2 and 4 h cyanide pulses stimulated respiration (33 and 35\% respectively) and germination (66 and 87\%, respectively) to a similar extent. Shorter pulses (30 and 60 min) also enhanced respiration (19 and 25\% respectively) but did not release dormancy (Tab. 12). Clearly, while the release of seed dormancy by cyanide is inevitably preceded by an increase in oxygen uptake, cyanide-induced respiration does not appear to be related to germination process(es) \textit{per se}.

Adkins \textit{et al}. (1984b,d) have recently reported that nitrate, nitrite and ethanol stimulate respiration of dormant wild oat seeds in
advance of germination. Whether or not these compounds have the same
mode of action is not known. The fact that several chemically
different dormancy-breaking compounds all stimulate pre-germination
respiration is suggestive of a causal relationship between the two
phenomena. However, a non-causal relationship between respiratory
elevation and the release of seed dormancy cannot be ruled out with
the available data.

E. The effect of SHAM on the release of dormancy by azide and cyanide.

Although SHAM is known to inhibit seed germination in the presence
of azide (Upadhyaya et al., 1983; Adkins et al., 1984 c,d) the
physiology of this inhibition is not understood. Upadhyaya et al.
(1983) suggested (as one possibility) that the inhibitory effect of
SHAM may be due to the complete inhibition of ATP production in the
presence of azide. Accordingly, alternative respiration is necessary
for supplying ATP in the presence of azide and may not be the cause of
dormancy release per se. The results in Tab. 10 suggest that SHAM
does not act in this manner. When 10 mM SHAM was present during and
after a 6 h azide pulse, the release of dormancy was inhibited;
however, if SHAM was applied only after the 6 h azide treatment
germination proceeded with little inhibition. Similarly when SHAM was
present only during the 6 h azide pulse, germination was not reduced.

If SHAM blocks azide-induced germination by halting ATP generation via
the alternative pathway one would expect SHAM applied after the azide
pulse also to inhibit germination since it is unlikely that sufficient
ATP could be generated in this 6 h period to support subsequent germination when both pathways are blocked. It is possible that SHAM applied during, but not after the azide treatment failed to inhibit germination because the seeds were not in contact with the chemical long enough to absorb an inhibitory dose or that SHAM taken-up by the seed is metabolized to an inactive form.

Given the similarity of cyanide and azide action on seed dormancy and respiration, it was unexpected to find that SHAM (applied concurrently) failed to inhibit the release of dormancy by cyanide as it had in the case of azide (Tab. 10). To test the possibility that the lack of inhibition by SHAM in the case of cyanide was due to slow uptake of SHAM by the seed, SHAM was applied to seeds 24 h before cyanide treatments as well as during cyanide treatment. However there was still no inhibition of germination (data not shown). It is therefore unlikely that the release of dormancy by cyanide in wild oat seeds is dependant on a SHAM-sensitive process (alternative respiration). The difference in the effect of SHAM on cyanide and azide action is in contradiction to the notion that the two inhibitors have the same mode of action in releasing seed dormancy.

F. The nature of azide and cyanide stimulated respiration.
1. The effect of SHAM on the stimulation of respiraton by azide and cyanide.

The SHAM-sensitivity of the induction of seed respiration by azide and cyanide paralleled the results found with the release of dormancy.
SHAM did not inhibit the induction of respiration by cyanide as it did in the case of azide (Tab. 11). This would suggest that the mechanism for the induction of respiration by the two compounds is different - azide action requiring a SHAM-sensitive process while cyanide action does not. If SHAM inhibits the induction of respiration by inhibiting alternative respiration (stimulated by azide) then cyanide treatment must not induce this pathway.

2. The nature of azide and cyanide induced respiration.

To further investigate the SHAM sensitivity of cyanide and azide induced respiration, the nature of the induced oxygen uptake (as opposed to the nature of the induction of respiration) was examined.

a) Alternative (SHAM-sensitive) respiration.

Surprisingly, after seeds were removed from dishes containing azide, induced respiration could not be inhibited by the subsequent application of SHAM (Tab. 13). Taken alone, these results might suggest that the respiration of these seeds was cytochrome-mediated (presuming the seeds recovered from the previous inhibition of this pathway). This however, does not appear to be the case since SHAM applied in combination with azide or cyanide subsequent to respiratory stimulation failed to inhibit cyanide-induced respiration and had only a small effect on azide-induced respiration (Tab. 13). In this situation one would expect both pathways to be blocked thus a shift
from the alternative pathway to the cytochrome pathway could not occur.

It was of concern that SHAM might be entering the seeds in the presence of azide but not when applied alone or in combination with cyanide due to effect(s) azide might have on membrane permeability. If this was the case, then this could explain the lack of inhibition of cyanide-induced germination and respiration by SHAM (Tabs. 10 and 11); inhibition of cytochrome-mediated respiration by cyanide might increase the flux of electrons through the alternative pathway but this would not be detected if SHAM is unable to penetrate the seed and inhibit alternative respiration. For this reason, SHAM was applied in combination with azide to seeds that had been pre-treated with cyanide or azide (Tab. 13). No respiratory inhibition resulted. Clearly, the SHAM-insensitivity of cyanide and azide-induced respiration cannot be due to the inability of SHAM to penetrate seeds. SHAM has been used in many seed physiological studies without any reported penetration problems (Esashi et al., 1981b, 1982; Yu et al., 1979; Yentur and Leopold, 1976). These results suggest that while the induction of respiration by azide appears to be a SHAM-sensitive process (Tab. 11), once induced, cyanide and azide-stimulated respiration are not SHAM-sensitive (Tab. 13) and thus not alternative.

The sensitivity of induced respiration to inhibitors was more accurately determined by partitioning the respiration of embryos excised from seeds previously treated with azide or cyanide (Tab. 16). As in intact seeds (Tab. 13), the actual contribution of the
alternative pathway was essentially negligible in controls, and in azide or cyanide treated seeds. Clearly, azide and cyanide treatment did not cause the activation of the alternative pathway.

b) Residual respiration.

It is clear from Table 16 that the stimulation of respiration by azide and cyanide is expressed through large increases in residual respiration. This source of oxygen uptake is generally considered to be nonmitochondrial in nature (e.g. Theologis and Laties, 1978a). Unfortunately, attempts to isolate mitochondria from seed tissues were not successful and therefore the possibility that some or all of the residual respiration was of mitochondrial origin could not be entirely discounted, although it is highly unlikely given our understanding of mitochondrial respiration pathways.

Two enzyme systems utilizing molecular oxygen (catalase and lipoxygenase) have lead to some confusion in interpreting respiratory data obtained in seed systems. Lipoxygenase (LOX) can account for a large proportion of total respiration in some tissues (e.g. soybean cotyledons, Theologis and Laties, 1978a). It is unlikely however that this is the case in azide/cyanide treated wild oat seeds since: i) LOX does not exist to an appreciable extent in wild oat seeds (Upadhyaya et al., 1983) and ii.) LOX is inhibited by SHAM (Parrish and Leopold, 1978) which failed to inhibit cyanide and azide induced respiration in wild oats (Tabs. 13 and 16).
Hendricks and Taylorson (1975) found that at low concentrations cyanide inhibited catalase but not respiration in seeds of several species. They attributed the promotion of germination by cyanide to elevated levels of peroxide. Others (Esashi et al., 1979a) have found no evidence of peroxide accumulation in cyanide or azide treated seeds. In wild oats it is unlikely that catalase inhibition could account for the observed increase in respiration upon cyanide or azide treatment. Since catalase activity evolves oxygen, catalase inhibition would be recorded polarographically as an increase in oxygen consumption by the seeds due to change in the concentration of dissolved oxygen. In this situation, oxygen uptake would be inhibited by cyanide since respiration would still be cytochrome-mediated. Table 16 shows however, that cyanide at concentrations sufficient to completely inhibit cytochrome-mediated respiration in embryos excised from water-imbibed seeds had little effect on the respiration of embryos excised from azide or cyanide treated seeds.

c) Cytochrome-mediated respiration.

Interestingly, cytochrome-mediated respiration of embryos excised from azide and cyanide treated seeds was similar to that of the control (Tab. 16), implying that 1 mM azide or cyanide treatment (under the given conditions) did not irreversibly inhibit cytochrome oxidase. Although it could be argued that during excision and stirring of the embryos in the oxygen uptake vessel, azide and cyanide
are leached from the tissue, this is improbable since, if the inhibitors were lost during the measurement of oxygen uptake then one would expect to see a gradual increase in respiration as the internal inhibitor concentration decreased rather than a constant rate of respiration as was observed. Additionally, in preliminary experiments where embryos were excised and placed in Petri dishes containing azide, respiration was inhibited and this inhibition did not decrease during the measurement of oxygen uptake.

Similarly, respiration induced by azide or cyanide treatment in intact seeds was not sensitive to 1 mM azide or cyanide (applied following the initial 24 h treatment - Tab. 13); in fact renewing azide or cyanide solution further stimulated oxygen uptake by an additional 30 - 40%.

Although concentrations of azide and cyanide used in this study give good inhibition of cytochrome-mediated respiration in most tissues (Theologis and Laties, 1978a,b), the seed coat may present a permeability barrier rendering the inhibitor concentrations used here insufficient to inhibit cytochrome-mediated respiration. Taylorson and Hendricks (1973) found that cyanide and azide did not inhibit cytochrome-mediated respiration at concentrations which promoted germination in dormant lettuce seed (0.1 mM).

G. The effect of pH on the action of azide and cyanide on seeds.

During the course of this study it was reported that the activity
of cyanide and azide on seeds was pH-dependent (Cohn, 1985). Azide and cyanide are weak acids (hydrazoic acid, $\text{HN}_3$ - pKa 4.7 and hydrocyanic acid, HCN - pKa 8.9 respectively - James, 1953) upon dissolution. Accordingly, decreasing pH will shift the molecule/ion equilibrium, increasing the proportion of undissociated molecules in the solution. It is the undissociated molecule that is able to passively diffuse through the phospholipid phase of the plasmalemma (Simon and Beevers, 1952). Consequently, an increase in the concentration of undissociated molecule in the external medium will result in a corresponding increase in the internal concentration of the inhibitor. Once in the cytoplasm, azide and cyanide both inhibit cytochrome oxidase as undissociated molecules (James, 1953). Thus decreasing external pH will enhance the inhibition of cytochrome oxidase by facilitating inhibitor diffusion into the cell by creating a steep concentration gradient and by increasing the concentration of the active (undissociated) form of the inhibitor within the cytoplasm.

1. The effect of pH on the stimulation of respiration by azide and cyanide.

Since dissolution of azide, cyanide and SHAM in distilled water results in substantial pH change (Tab. 17), it was of concern that some of the effects of the respiratory inhibitors could be due in part to indirect effects of pH changes rather than to direct effects of these compounds on respiration. Experiments examining pH effects were thus carried-out. Although seed respiration per se was unaffected by
the pH of the imbibitional medium (pH 5-8, Figs. 12 and 13), the
effect of azide on seed respiration was strongly pH-dependent (Fig.
12). At pH 5 and 6, azide concentrations greater than 0.0625 and 0.25
mM, respectively, not only failed to stimulate seed respiration (as
occurred at pH 7) but actually reduced oxygen uptake far below that of
the control. Lower concentrations of azide were effective at
stimulating respiration at pH 5 and 6.

Simon and Beevers (1952) found that the inhibition of respiration
by hydrogen fluoride (pK_a = 3.2) was extremely pH sensitive. They
found that inhibition of respiration by this compound could be altered
by i) maintaining a constant concentration while altering pH or by ii)
changing inhibitor concentration while maintaining a constant pH (the
inhibition curves for both were sigmoidal). The authors concluded that
it was the undissociated HF molecule that inhibited respiration since
low pHs increased its inhibitory activity. An analogous situation
appears to exist with azide activity on seed respiration. It seems
highly likely that the inhibition of respiration by azide (Fig. 12) is
due to inhibition of cytochrome-mediated respiration, implying that
azide concentrations stimulating seed respiration do not inhibit
cytochrome oxidase. At pH 5 the undissociated azide concentration
would be higher than at pH 6, thus where 0.25 mM azide stimulates
respiration at pH 6, at pH 5 this concentration is high enough to
inhibit cytochrome oxidase and thus oxygen uptake. At pH 7, azide did
not inhibit seed respiration at concentrations as high as 2.0 mM;
clearly azide is a much less potent inhibitor of cytochrome oxidase at
this pH.
In contrast to azide, the induction of respiration by cyanide was not strongly effected by change in pH (5-7, Fig. 13). This is expected since pH values used in this experiment were all below pKa for cyanide (8.9), consequently the external equilibrium was strongly in favor of the undissociated HCN molecule. At low concentrations (0.065 to 0.25 mM) cyanide activity was enhanced somewhat by low pH. However, this effect disappears at higher cyanide concentrations where the cyanide concentration was already sufficiently high to give maximum respiratory stimulation. The failure of cyanide to inhibit respiration at even the lowest pH and highest concentration may be due to the gradual loss of cyanide from the treatment plates (Yu et al., 1981). Consequently cyanide concentration may not have remained high enough for sufficient time to inhibit cytochrome-mediated respiration. This is probable since low pHs are known to favor the formation of gaseous HCN (Merck Index, 1983). Applied at pHs exceeding the pKa value of cyanide, it is likely that a decrease in effectiveness (at stimulating seed respiration) would have been observed at low concentrations.

2. Comparison of the effect of SHAM and pH on the action of azide and cyanide.

Further examination of the effect of pH and buffering on azide activity revealed that at pH 5, 1 mM azide inhibited respiration to the same extent as 1 mM azide + 10 mM SHAM in distilled water (pH 5.2 - Tab. 17). This suggests the possibility that the inhibitory effect of SHAM on the induction of respiration by azide in an unbuffered
system may be an artifact related to its capacity to acidify the solution (Tab. 17) (thus increasing the concentration of undissociated azide) rather than through the inhibition of SHAM-sensitive processes (including alternative respiration). It is possible that SHAM lowers pH to an extent such that the undissociated azide concentration is increased to the point where 1 mM azide is sufficient to inhibit cytochrome-mediated respiration in imbibing seeds (i.e. as in Fig. 12). This hypothesis would explain why, when buffered at pH 7, SHAM (applied simultaneously) had no effect on the stimulation of seed respiration by this compound (Tab. 17). To test the hypothesis that SHAM effects the stimulation of respiration by azide in the same manner as declining pH, the effect of SHAM and pH on the induction of seed respiration by different concentrations of azide were compared (Fig. 14). If the effect of SHAM on the induction of respiration by azide is purely a pH effect then SHAM should effect the response of seeds to various azide concentrations differently since respiratory stimulation occurs only within a narrow concentration range of azide (Upadhyaya et al., 1983; Fig. 12). Concentrations of azide too low to increase respiration would become stimulatory since the concentration of the undissociated molecule would increase while concentrations stimulating respiration could become inhibitory as the concentration of undissociated inhibitor becomes sufficiently high to inhibit cytochrome oxidase. The results in Fig. 14 show this to be the case. Azide (in distilled water) was effective in stimulating respiration at concentrations of 0.75 and 1.0 mM; SHAM (10 mM) inhibited this
stimulation. However, lower concentrations of azide (0.1 - 0.25 mM) that alone did not stimulate seed oxygen uptake, increased respiration in the presence of 10 mM SHAM (Fig. 14). This can be explained by pH changes. In distilled water, 0.25 mM azide is sub-optimal for respiratory stimulation (Fig. 14), however when applied with 10 mM SHAM the solution pH declines (Tab. 17) resulting in concentration of undissociated azide high enough to give maximal stimulation of oxygen-uptake. At azide concentrations higher than 0.25 mM, azide + 10 mM SHAM cause respiration to be inhibited (Fig. 14). Here declining pH results in dissociated azide concentrations sufficiently high to completely inhibit cytochrome oxidase. Direct comparison of the effect of SHAM and of pH (altered such that the pH of each azide solution was equal to that of azide + SHAM at a given azide concentration) on azide action supports this hypothesis. When the pH of an azide solution was adjusted to mimic the drop in pH incurred with simultaneous dissolution of 10 mM SHAM, the change in azide activity was practically identical to that observed with concurrent application of 10 mM SHAM. The displacement of the azide (pH altered) and azide + SHAM curves along the abcissa (Fig. 14) could be due to small changes in pH occurring in the unbuffered azide + SHAM solutions over the 24 h incubation period, which would in turn, affect azide activity.

Given the lack of sensitivity of the induction of respiration by cyanide (Fig. 13) at low pH, it was not surprising that 10 mM SHAM applied concurrently with cyanide had no effect on the induction of
respiration by cyanide regardless of its concentration (Fig. 15). Any increase in the concentration of undissociated cyanide afforded by SHAM's effect on medium pH was not significant with respect to the activity of cyanide on seed oxygen consumption.

It is known that the undissociated and the ionic forms of azide and cyanide have different effects on cell physiology. For example, while the undissociated molecules of azide and cyanide inhibit cytochrome oxidase, the ions of both inhibit cytochrome c (James, 1953). The dual activity of azide and cyanide on wild oat seeds (i.e., stimulating and inhibiting seed respiration) could conceivably be explained by the differing actions of the ions and molecules of these inhibitors. However, since decreasing pH (by SHAM addition, Fig. 14) enhances the stimulation of respiration by low concentrations of azide and inhibits it by higher concentrations, the undissociated molecule of azide and cyanide appears to be responsible for both effects.

3. The effect of pH on the release of seed dormancy by azide and cyanide.

Upadhyaya et al., (1983) found that when azide was given to seeds at concentrations high enough to completely inhibit respiration, the release of dormancy (and/or germination) was also inhibited (Upadhyaya et al., 1982a). Similarly, cyanide at concentrations high enough to inhibit respiration does not stimulate germination (Fig. 10). The inhibition of germination by 1 mM azide + 10 mM SHAM is undoubtedly
due to the increase in azide concentration afforded by SHAM; not necessarily by blockage of the alternative pathway. This is supported by the lack of germination of seeds treated with 1 mM azide at pH 5 where no blockage of the alternative pathway occurs (Tab. 18). Additionally, at pH 7, 10 mM SHAM failed to inhibit the release of dormancy by 1 mM azide (data not shown). The failure of SHAM to inhibit the release of dormancy by cyanide can also be explained by pH. Since the activity of cyanide in releasing seed dormancy is not strongly pH dependent (pH range 5 to 8, Tab. 19), acidification of the media that occurs when SHAM is dissolved will not deleteriously affect the ability of cyanide to promote seed respiration. The release of seed dormancy by cyanide, as with the stimulation of seed respiration, does not require alternative respiration.

It appears therefore, that the stimulation of respiration and germination by cyanide and azide requires cytochrome-mediated respiration regardless of whether or not the alternative pathway is blocked by SHAM. This can be deduced from Tables 17 and 18. When azide is applied to seeds at concentrations high enough to inhibit cytochrome-mediated respiration (i.e. 1 mM at pH 5, Tab. 17), the stimulation of respiration is prevented as is the release of seed dormancy (Tab. 18). Thus, it appears that the mechanism(s) whereby respiration and germination are stimulated is not related to the inhibition of cytochrome oxidase by azide and cyanide.
H. General discussion.

What then, is the status of the alternative pathway in wild oat seeds? Although alternative respiration does not appear to be involved in the regulation of seed dormancy in wild oats, intact seeds (as well as excised embryos, Tab. 6, 16) have the ability to divert electrons via this pathway when the cytochrome pathway is blocked. This can be deduced by examining Tables 14 and 15. Azide (5 mM) + 10 mM SHAM inhibited respiration more than 5 mM azide alone. Since in the absence of SHAM, increasing azide concentration beyond 5 mM did not further inhibit respiration (Tab. 14) it can be concluded that maximum inhibition of cytochrome respiration occurs at 5 mM. Thus SHAM did not increase the inhibition of respiration by 5 mM azide by causing a pH decline and a consequent increase in the undissociated azide concentration. Therefore, alternative respiration can occur in wild oat seeds when the cytochrome pathway is completely blocked; the maximal flux of the alternative pathway in these seeds is however, only 15–20% of the CN-sensitive pathway (Tab. 16). Others have also found a similarly low capacity of the alternative pathway in seeds (Miller et al., 1983).

Clearly, the interpretation of results obtained using SHAM as an inhibitor of alternative respiration must be made with caution. Not only does SHAM inhibit enzymes such as lipoxygenase, peroxidase and tyrosinase (Rich et al., 1978) but a recent study (Upadhyaya, 1986) shows that SHAM at concentrations frequently used in seed studies inhibits the GA₃-induced α-amylase production and the subsequent release of reducing sugars. The inhibitory effect of SHAM alone on
seed germination (Yentur and Leopold, 1976; Yu et al., 1979; Esashi et al., 1980) may not be entirely due to the inhibition of the alternative oxidase but may involve some secondary effects as well.

The results of this study highlight the need to challenge several assumptions often made in studies involving seeds and respiratory inhibitors. Cyanide and azide concentrations frequently employed to inhibit cytochrome oxidase in bulky sliced tissues (Theologis and Laties, 1978 a,b) may not be sufficient to inhibit cytochrome oxidase in intact seed. Levels of cyanide and azide stimulating respiration and germination do not appear to inhibit cytochrome-mediated respiration in wild oat seeds; conclusions based on seed studies using similar inhibitor concentrations must be reevaluated (Adkins et al., 1984c,d; Zagerski and Lewak, 1983). When using azide and cyanide at concentrations known to effect other enzymes (i.e. formic dehydrogenase, uricase, catalase and peroxidase; James, 1953), caution must be taken not to attribute all of the effects of these inhibitors to the inhibition of cytochrome oxidase.

Respiratory inhibition by azide in the presence of SHAM when neither compound inhibits respiration individually (in an unbuffered system), may erroneously be taken as evidence of alternative respiration occurring in the presence of azide alone. Adkins et al. (1984 c,d), using an unbuffered system, found that neither 1 mM azide or 10 mM SHAM inhibited the induction of respiration by ethanol and nitrate in wild oat seeds. However, when azide and SHAM were applied in combination, respiration was strongly inhibited (>90%). The authors explanation was as follows: ethanol and nitrate ions can
stimulate seed respiration via the cytochrome or the alternative pathway, either providing the ATP necessary to support germination. When azide (1 mM) is applied simultaneously with ethanol or nitrate, respiration proceeds via the alternative pathway; on the other hand, when SHAM (10 mM) is applied concurrently with ethanol or nitrate, respiration is entirely cytochrome-mediated. Consequently, when both azide and SHAM are applied with nitrate or ethanol, respiration cannot proceed because both respiratory pathways are blocked. In light of the results of the present study, it seems highly probable that the respiration induced by nitrate and ethanol was inhibited by a combination of azide and SHAM by virtue of complete blockage of the cytochrome pathway (i.e. by increasing the undissociated azide concentration) and that any blockage of the alternative pathway was immaterial to the observed respiratory inhibition. If this explanation is correct, then it appears that, as with azide and cyanide, cytochrome-mediated respiration is probably necessary for the stimulation of germination and respiration by ethanol and nitrate.

Adkins et al. (1984 c,d) failed to distinguish between the nature of the induction of respiration by nitrate and ethanol and the nature of the induced respiration. Although azide and SHAM applied simultaneously with ethanol or nitrate inhibited respiration, whether azide, SHAM or a combination of both would have inhibited the stimulated oxygen uptake (i.e. applying respiratory inhibitors subsequent to the application of nitrate or ethanol) was not studied.
Given the similarity (i.e. very similar time course and sensitivity to respiratory inhibitors) of the stimulation of wild oat seed germination and respiration by azide, cyanide, ethanol and nitrate it seems highly probable that the origin of oxygen uptake stimulated by these chemically diverse compounds is the same. If this is the case it would be tempting to put forth the hypothesis that while the mode of action of these chemicals in releasing seed dormancy might differ, their direct or indirect stimulation of some nonmitochondrial source of oxygen-consumption might ultimately be responsible (or necessary) for the subsequent release of seed dormancy in wild oats.

At this time the nature of the induced, nonmitochondrial oxygen-consumption remains a mystery. It would be valuable to pinpoint its source in order to further establish a link between it and the release of seed dormancy in wild oats and in other species.
CONCLUSIONS

A. The induction and release of secondary seed dormancy.
   1. As with primary dormancy, pure lines (both nondormant and
dormant) of wild oat exhibit genetic variability in their
secondary dormancy behaviour and factors like temperature can
modify the expression of this trait.
   2. Treatments effective at releasing primary dormancy (i.e.
chemicals, after-ripening) also released secondary dormancy,
suggesting that the two types of dormancy are similar, at least in
part, in their regulation.
   3. Alternative (SHAM-sensitive) respiration is not involved in
regulating the induction or release of primary and secondary
dormancy.

B. The effect respiratory inhibitors on seed respiration and the
release of dormancy.
   1. Azide and cyanide stimulate seed respiration and germination at
similar concentrations and treatment durations and appear to act
by the same mode of action. Cyanide, however, was more effective
at releasing dormancy in freshly harvested seeds; azide was
impotent at releasing primary dormancy in these seeds.
   2. Although the release of dormancy by cyanide is always preceded by
an increase in seed oxygen consumption, a causal relationship
between the two could not be established.
   3. The induction of seed respiration by azide (but not by cyanide)
was completely inhibited by simultaneous application of SHAM.
However, once induced, both azide and cyanide stimulated
oxygen consumption were insensitive to SHAM. Thus, the stimulated respiration is not alternative and was found to be residual in nature.

C. The effect of pH on the stimulation of respiration and germination by respiratory inhibitors.

1. The action of azide on seed respiration and germination is extremely pH-sensitive. Decreasing pH increases the concentration of undissociated (active) azide which in turn effects the action of azide on seeds. A single concentration of azide can be either stimulatory or inhibitory to respiration and germination depending on the pH of the solution. Evidence suggests that azide and cyanide at concentrations stimulating seed respiration and germination, do not inhibit cytochrome oxidase.

2. Inhibition by SHAM of the stimulation of respiration and germination by azide was due to acidification of the medium upon dissolution of 10 mM SHAM. This increases the concentration of undissociated azide molecules, resulting in inhibition of both cytochrome oxidase and consequently, germination. Any blockage of the alternative pathway is incidental to the failure of azide to release seed dormancy or stimulate respiration in the presence of SHAM.

3. The stimulation of respiration and germination by azide and cyanide appears to require cytochrome-mediated respiration regardless of the occurrence of the alternative pathway.
BIBLIOGRAPHY


