Effects of Treatment With Different Concentrations of Oxygen on Germination of Sugarcane (Saccharum Officinarum) and Nutgrass (Cyperus Rotundus).

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EFFECTS OF TREATMENT WITH DIFFERENT CONCENTRATIONS OF OXYGEN ON GERMINATION OF SUGARCANE (SACCHARUM OFFICINARUM) AND NUTGRASS (CYPERUS ROTUNDUS)

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Botany and Plant Pathology

by

Narendra Singh Negi
B.S., Agra University, 1949
M.S., Banaras Hindu University, 1951
August, 1962
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ABSTRACT

Yield of Louisiana sugarcane could be considerably increased with improved germination. Factors affecting germination are often difficult to study under normal environmental conditions, but extreme variations of environment, such as the complete absence of oxygen or use of pure oxygen atmosphere might facilitate study of these factors. This investigation was concerned with the effect of oxygen concentration on the buds of sugarcane (*Saccharum officinarum*) and nutgrass (*Cyperus rotundus*).

The effects on the germination of full and half length stalks, single bud cuttings and isolated buds of sugarcane treated with 0, 20, 60 and 100 per cent oxygen for 24 and 48 hours were studied under field, greenhouse, and laboratory conditions. Parallel studies of nutgrass tubers were conducted in the laboratory. Only the 0 per cent oxygen and 100 per cent oxygen treatments caused consistent effects.

Compared to 100 per cent oxygen the 0 per cent treatment improved early germination of single bud cuttings, but the final germination with the former treatment was superior. Increased duration of both the treatments increased germination of single bud cuttings from the entire stalk, more so in the 0 per cent oxygen treatment, but the increase was less in buds from the lower stalk only.

In half length stalks germination was higher throughout the germination period in the pure oxygen treatments. Germination increased with the treatment duration with 0 per cent oxygen treatment.
It is proposed that the effects of pure oxygen supplement those of apical dominance in lower buds but lessen the effects in upper buds.

With intact stalks, germination was best following the 0 per cent oxygen treatment.

Laboratory experiments indicate that "germination factors" dependent upon vegetative tissue was influenced by the 0 per cent oxygen treatment while completion of the germination was influenced by the pure oxygen treatments.

Oxygen uptake by sugarcane buds increased with increasing oxygen concentration.

In nutgrass tubers germination was stimulated by short exposure and inhibited by long exposure to pure oxygen. Influence of apical dominance on effects of 100 per cent oxygen treatments was noted.

Spectrophotometric studies of neutral tuber extracts revealed an absorbance curve similar to that of caffeic acid-indoleacetic acid, but the identification was not positive.

Indications drawn from the data are that the 0 per cent treatments affect a quantitatively larger "germination factor" than the pure oxygen treatments, which possibly affect an auxin or other substance of small bulk or influence the interaction of auxin and kinetin.
INTRODUCTION

Yield of any crop is dependent on the density of the population of the crop plant and the vigor of the individual plants forming the population. When the population of plants is below a certain minimum the yield can hardly be increased by increasing the vigor of the plants. Above this minimum limit it is often easier to increase the yield by increasing the density of population than by making improvement in the vigor of the plant.

One reliable way to achieve a high density of plant population is by obtaining a high and uniform germination of the crop. Increasing crop yield through improvement in germination is advantageous because it is relatively easy and economical to improve germination by pretreatment of seed material. It is thus evident that among the various phases of life cycle of a crop the germination phase occupies an important place.

In crops like sugarcane, where a large part of the crop has to be used for "seed" and germination is rather poor, the study of germination process assumes special significance. Though numerous studies of germination of sugarcane cuttings have been made, a clear picture of the basic factors involved in germination has not been obtained so far.

The process of germination involves a number of factors, some of which are easily influenced by normal fluctuations in environmental conditions, while others are not materially affected.
example, the moisture content of the sugarcane cutting will be influenced by a lowering of soil moisture, but this will not materially affect the auxin or nucleic acid content of the tissue. The role of such relatively stable factors is somewhat difficult to assess under normal environmental conditions because of interaction of the effects of the various factors concerned in germination. On the other hand, under extreme variations of environmental conditions the basic role of some of these factors may be revealed.

An attempt has been made along these lines in the present investigation by studying the germination of sugarcane and nutgrass buds after subjecting them to different atmospheric conditions varying from complete absence of oxygen to a pure oxygen atmosphere.
Germination in Sugarcane

Germination* of sugarcane has been reviewed by Dillewijn (1952) and Burr et al (1957). Most of the investigations in this field are concerned with methods of improving germination rather than the basic study of the germination process.

The existence of a germination gradient along the stalk is the characteristic feature in sugarcane. The buds at the morphological top germinate more readily than those from the lower section. Various internal factors such as water, nutrient and growth regulating substance have been considered to govern this expression of gradient.

It has been shown that parallel with germination, moisture (Clements and Kubota, 1942), glucose (Went, 1894) and nitrogen (Clements and Moriguchi, 1942) decrease from the top towards the base while sucrose decreases in the reverse direction.

There is no direct evidence for the role played by growth regulating substances but it has been inferred from what is known about the phenomenon of apical dominance (Dillewijn, 1952). Although the germination can be explained on the basis of the phenomenon of apical

*The term germination should only be used for development of a plant from the seed embryo but the term "germination" for development of buds in sugarcane and nutgrass has been used here in view of the widespread usage of the term in the literature.
dominance for greater length of stalk the germination of the buds of
the basal part of the stalk often show an increase towards the lowest
buds which are located near the soil surface (Bonazzi, 1928 and Brandes
and Overbeek, 1948).

The earliest studies on metabolic changes in the germinating
bud and internode were made by Went in 1894. He found perceptible
changes in glucose and starch content in internode and bud one to
two days after planting. His studies suggest that glucose, alone
or with sucrose, flows from the cutting to the developing bud, some
of the sugars being stored in the form of starch around the canals
in which they move. Glucose is partly accumulated in the elongating
parts but is absent from the growing point. In contrast much starch
and albumin are found in the growing point and less in the cell
elongation region. An interesting observation concerned the increase
in tannin content some five to eight days after planting, when it
accumulates gradually in the growing point. As soon as the joints
become visible to the naked eye, they no longer contain tannin. He
suggested that the developing bud exerts an influence on the cutting,
resulting in conversion of sucrose into glucose especially in the
neighborhood of the bundles. Glaziou (1958) studied nutritional and
physiological relationship in germinating cuttings under conditions
when all nutritional requirements of the growing region were obtained
from the cutting itself. The germination of buds was accompanied
by a marked increase of moisture in the first seven days, after which
there was a gradual decrease and a movement of nutrients from the
cutting to the growing region. Virtually all of the nitrogen of the
bud before germination was in the alcohol insoluble form but both
alcohol soluble and insoluble nitrogen increased with time. He found that all single bud cuttings germinated when an external water supply was available, but it was only sporadic if the cuttings were placed merely in an atmosphere saturated with water. On the basis of these findings and known effects of auxin he suggested the following changes in a bud germinating without external water supply. First the destruction of some of the native auxin would free the bud of an inhibitory effect and might result in increased plasticity of the cell walls. The water relationship of the bud to the set may then allow a movement of water from the set and cause expansion in the bud of a sufficient degree to trigger cell division in meristematic regions. If the physiological conditions in the bud do not allow a movement of water from set to bud despite increased wall pressure, germination would occur only if an external water supply were available. His findings show that initiation of germination was accompanied by a movement of nutrients from the cuttings to the growing region. The transport of sugar from the cuttings appeared to be limited by a first order chemical reaction and not by simple diffusion. He agreed with Went's (1894) suggestion regarding influence of the bud and further hypothesized that the influence exerted by the bud may either represent a movement of material along a concentration gradient or it may actually secrete substances which may bring about mobilization of the nutrients available in the set.

Growth regulating substances have been extracted from nodal regions of sugarcane (Overbeek et al, 1945, Engard and Nakata, 1947 and Vlitos and Cutler, 1960). The substances extracted by Overbeek et al (1945) appeared to be of the type of indoleacetic acid rather
than auxin a or auxin b. They also observed that the nodes showed marked response to geotropic stimulation, however, the characteristic unilateral distribution of growth substances in the growth ring was absent after the stalks have been in horizontal position for a few days. They interpreted their results as indicating that a factor other than auxin is the controlling agent in the growth and geotropic reaction of the sugarcane node.

Overbeek et al (1945) also reported that a growth inhibiting substance from nodal regions of sugarcane was released as a result of immersion of sugarcane material in boiling water for a brief period. The substance was water and ether soluble.

Engard and Nakata (1947) found that the ether fraction of neutralized water extract of sugarcane produced a negative curvature of oat coleoptile while the ether fraction of acidified water extract gave mostly positive curvature. These findings led them to suggest the presence of at least two substances, one a growth promotive substance of acid type and second an inhibitor type which inhibited elongation of coleoptile either directly or indirectly by effect on growth promoting hormone.

Overbeek (1943) showed that auxin application on sugarcane promoted the development of roots from the root band and inhibited the lateral buds. Khan and Hall (1954a) found that those concentrations of indoleacetic acid and α-ortho-chlorophenoxy propionic acid (α-o-CPA) which favored root development generally limited germination of buds. However, a mixture of the two in proportion of 1:9 tended to mask the inhibitive effect on the bud or buds. In another experiment (Khan and Hall, 1954b) combined use of indoleacetic acid and α-o-CPA
with sucrose and potassium permanganate, respectively, resulted in significantly greater germination. According to these authors this increased germination was probably due to an accelerating effect of sucrose on movement of indoleacetic acid and possibly reduction of 4-0-CPA toxicity through oxidizing action of potassium permanganate. They also found that cuttings from the top portion responded to added sucrose and those from the basal portion to auxin, suggesting thereby the necessity of suitable auxin sugar balance for optimum response.

Various methods for enhancing germination have been tried. Of these soaking in cold water (Kamerling, 1900) and warm water (Brandes and Klapkaak, 1923), soaking in lime solution and various fertilizer solutions (Evans, 1933), soaking in 10 per cent ethyl alcohol, treatment with chlorhydrin and acetylene (Overbeek, 1944) and treatment with light (Rao and Sinha, 1954) are of interest.

The beneficial effect on germination of soaking in water and lime solutions was ascribed, by many workers, to the increase in moisture content of the cutting or conversion of carbohydrates into reducing sugars. Since cuttings altogether fully saturated with moisture may respond favorably to soaking in water the increase in the moisture content of the cutting may not be the reason for favorable response in all cases (Dillewijn, 1952). Brandes and Overbeek (1948) suggested that promotion of bud growth by soaking in hot water was due to leaching and consequent lowering of auxin content. Khan and Hall (1954a) consider that the improvement in germination following soaking in water may be either due to leaching of inhibitors from the cuttings or due to liberation of auxin activated by inhibition of water.
Germination in Nutgrass Tubers*

Though nutgrass is an important weed in many parts of the world, few basic studies have been made with regards to germination of its tuber which is the chief means of its propagation.

Andrews (1946) noted in one of his experiments that about three per cent of the tubers did not germinate, there being no apparent causes like physical damage, immaturity or insect damage, etc. He thought that these tubers did not germinate because of damage to their root structure. Palmer and Porter (1959a) studied germination of nutgrass as influenced by oxygen levels. These studies showed that when tubers were exposed to 1, 10 and 20 per cent oxygen in mixture with 1 per cent carbon dioxide the percentage germination was 34, 70, 77, respectively. As the carbon dioxide concentration was increased, germination decreased in 1 and 10 per cent oxygen and slightly increased in 20 per cent oxygen. Ninety-five per cent of single tubers germinated in 1 per cent oxygen. When chains of tubers were exposed to these gas mixtures more tubers germinated in the higher oxygen levels than in the lower levels. When dormant tubers were separated from a chain in which certain tubers were germinated and exposed to air, they germinated, indicating that the germinated tubers exerted dominance in the chain.

*Morphologically the underground swollen part of the stem in nutgrass is a corm but the term "tuber" has been used here following the general usage in literature.
Respiration in Sugarcane and Nutgrass

No investigations on respiration seem to have been made with particular reference to the germination process in sugarcane. Bonazzi (1931) investigated the gaseous composition and made a rough estimation of localization of oxidizing enzymes in sugarcane stalks. He found that oxygen was more abundant in the basal portions of sugarcane and showed a sharp decrease towards apical regions while CO\textsubscript{2} content showed just the opposite trend. Oxidizing enzymes decreased from top to bottom. He hypothesized a close connection between these changes and germination gradient. Bieleski (1958) investigated respiratory changes in harvested sugarcane stalks. He found that the respiration rate, measured as carbon dioxide evolved, was only 12 per cent higher in 100 per cent oxygen than in air, whereas in 5-6 per cent oxygen it was 33 per cent lower and in nitrogen 60 per cent lower. In cane stored in air there was practically no alcohol and 25 mg/kg of fresh weight of volatile organic acids were present. In contrast the cane stored for 48 hours in nitrogen showed a very high alcohol production and slightly higher volatile organic acids. No aldehyde could be detected. He found that the calculated rate of alcohol formation corresponded closely with the known rate of anaerobic carbon dioxide production in sugarcane. Carbon dioxide production in intact stalks showed increase after harvesting, reaching a peak value at 24 hours, and then decreased. Single internodes showed higher carbon dioxide production and also a second peak near about 120 hours. Khan and Hall (1954b) showed that the rate of respiration was accelerated by auxin treatment as the distance increased from the stalk apex.
Respiratory changes with regard to the germination of nutgrass tubers have been reported by Palmer and Porter (1959b). Dormant tubers showed an R.Q. of 2.1; however, oxygen consumption increased during germination and an R.Q. of unity was obtained in five and one half days. Total gas exchange increased as germination proceeded. Isocitric dehydrogenase associated with particles was more active in dormant tubers than in germinated tubers while succinic dehydrogenase was more active in germinated tubers. "Particles" from dormant tubers oxidized isocitrate, α-ketoglutarate and succinate. Malonate inhibited the α-ketoglutarate by 62 per cent. Catechol oxidation by the dormant tuber was 5.9 times higher than ascorbate oxidation. As germination proceeded, ascorbate oxidation increased with time, and at about five and one half days the oxidation of ascorbate and catechol were equal. On the eighth day ascorbate oxidation was 3.9 times greater than catechol oxidation. Germinating tubers showed 2.5 times higher peroxidase than dormant tubers. Respiratory response of tubers under different oxygen levels was also studied. Dormant tubers showed increased respiration with increase of oxygen levels from 1 to 100 per cent. The same was true for the germinated tubers; however, the total gas exchange was greater from the germinated tubers. The R.Q.'s for the dormant tubers were 2.9 and ½.5, respectively at the 20 and 100 per cent oxygen levels, but they were unity for the germinated tubers at these oxygen levels. The R.Q.'s for dormant and germinated tubers were indicative of anaerobic respiration in 1 per cent oxygen and to a lesser degree at 10 per cent level.
Internal Factors Concerned in Germination

Internal conditions of the bud causing inhibition of germination may be due to factors arising outside the bud as in case of correlative inhibition or the inhibitory conditions may prevail due entirely to factors arising in the bud itself.

Of the many mechanisms suggested to explain the phenomenon of correlative inhibition the one suggesting it as a direct function of auxin produced by the apical bud is most generally accepted. LeFanu (1936) has, however, reported that heteroauxin applied to the base of pea stem cuttings inhibited the bud above the site of application. Snow (1937) also suggested that bud inhibition may not be caused directly by auxin. Offering an explanation for the fact that the growing leaves near the apex tend to inhibit the lateral bud but promote growth of their own shoot he suggested that in a stem, in which auxin is traveling down from the growing leaves, the primary positive effect of the auxin overrides the secondary inhibiting effect; however, not much auxin travels in the morphologically upward direction into a lateral bud shoot whereas inhibitive influence does travel up into a lateral bud and so produces its effect there.

Skoog and Miller (1957) showed that in cultures of pith of Nicotiana tabacum, kinetin allows the production on one tissue culture fragment of very large numbers of buds and since these elongate side by side it indicated that they were not inhibiting development of one another. Wickson and Thimann (1958) found that the inhibition of lateral buds of isolated pea stem section by physiological concentrations of auxin was completely removed when kinetin at concentrations between 1 and 10 ppm were applied together with auxin. They suggested
that the normal phenomenon of apical dominance depends on an interaction between auxin and kinetin-like substance. In a more recent paper Wickson and Thimann (1960) reported that the indoleacetic acid content in the pea stem tissue could be reduced by (a) exposure to light, (b) simultaneous treatment with kinetin, or (c) by decapitation of the plant some days before the sections were cut. All three of these conditions also favored bud growth. Using isotopically labeled indoleacetic acid they also obtained evidence that indoleacetic acid is secreted in the apex, transported down the stem and up to lateral buds and thus may be a prime causal factor in maintenance of apical dominance.

Most of the studies with regard to the inhibition due to factors arising within developing tissue itself have been made with buds of trees, potato, and seeds. Meyer and Anderson (1956) think that it is probable that germination inhibitors are present in seeds of many species and they may represent a widespread mechanism of dormance in seeds. Veledestra and Havinga (1945) have mentioned certain unsaturated lactones which appear to be widely distributed and possess the property of inhibiting seed germination. Galitz and Howell (1959) described a natural germination inhibitor in premature soybean seeds which appeared to retain its inhibitory activity in crude natural or basic extracts and absorbed maximally at 260 μ. Miyamoto et al (1961) extracted four germination inhibitive fractions from wheat varieties with red kernel which were more resistant to germination. Two of these, designated as catechin-tannin fractions, were soluble in water but insoluble in chloroform. The other two were soluble in chloroform.
Substances considered to inhibit sprouting of buds have been shown to occur in buds of potato (Hemberg, 1949a), *Fraxinus* (Hemberg, 1949b), peach (Blommaert, 1955) and *Cyperus esculentus* (Tumbleson, 1960). The effect of these inhibitory substances in *Fraxinus*, peach and potato was inferred from the fact that they disappeared when the rest of the buds were broken, regardless of whether this took place naturally or was brought about by treatment with ethylene chlorhydrin or other means. Buch and Smith (1959), however, doubt the validity of these conclusions for potato, as according to them the inhibiting capacity of the substances was assayed by avena coleoptile tests. They used potato buds in a bioassay along with avena coleoptile straight growth test for testing inhibition of sprouting of potato buds. No delay in sprouting was found when eye pieces were planted in sand and in contact with inhibitor-containing segment of chromatogram. However, extraction of these eye pieces caused suppression of coleoptile growth, thus proving that the applied inhibitor was present in the eye pieces, although no delay in sprouting had occurred.

A germination inhibitor was also found in tubers of *Cyperus esculentus* which usually shows less than 10 per cent germination when harvested in fall (Tumbleson, 1960). However, if the tubers are thoroughly washed in cold water the germination increased up to 75 per cent. Water extract from tubers was found to inhibit germination of nutgrass tubers and seeds of alfalfa, peas, soybean, barley and oats. The inhibitory substance was heat stable, dialyzable, not adsorbed by charcoal or an ion exchange resin, was soluble in methanol and had an *Rf* between .95 and 1.0 when chromatographed in

Chemical Changes Associated with Germination

Gutherie (1933) showed that chemicals effective in breaking dormancy of potato buds also increased glutathione content significantly. However, a quantitative relation could not be shown between the effectiveness of a chemical treatment and its capacity to increase glutathione.

Miller et al (1936) indicated that increase in glutathione may play a part in the breaking of dormancy in potato by non-polar compounds such as ethylene chlorhydrin. The effects on respiration, pH, citric acid content, etc. were not considered important because these were affected in the same manner by butyl halides which were less effective in breaking dormancy than ethylene chlorhydrin. In contrast butyl halides decreased glutathione content. They further suggested that decrease in sulphate that followed treatment with ethylene chlorhydrin may be due to utilization of sulphate in the synthesis of glutathione. Samish (1954) in his review points out that the two rest breaking agents, thiocyanate and thio-urea do not cause synthesis of glutathione though they do contain bivalent sulfur.

Kastle and Clark (1903) found that unsprouted potato lacked invertase activity. Schiwimmer et al (1961) confirmed these findings and presented data which suggested that the difference in invertase activity between sprouted and non-sprouted potatoes may not be due entirely to a difference in actual invertase content of the tuber but may reflect the influence of presence of naturally occurring inhibition
of invertase activity in non-sprouted potatoes.

Skoog (1954) suggested that inhibition of lateral buds was presumably due to the inhibitory effect of endogenous auxin from the terminal bud on the ability of the promeristem cells of the lateral buds to produce DNA and to undergo mitosis and cell division.

Boresch (1920) considered that the effectiveness of warm bath treatment in breaking the rest of buds was dependent on a combination of raised temperature and anaerobic conditions and that products of anaerobiosis were the active agents in breaking rest.

Rakitin and Suvorov (1935) recommended anaerobiosis, caused by storing potatoes in absence of oxygen, for breaking dormancy of potato. Thornton (1939, 1944) showed that dormancy of freshly harvested potatoes could be broken in 7 days if they were held under 2 per cent oxygen under dry conditions or if held in 5 to 10 per cent oxygen under moist conditions at 23°C to 28°C. Bud growth was produced in tubers stored in pure nitrogen for 18 days; whereas dormancy under normal 20 per cent oxygen was broken only after 47 days. Higher percentages of oxygen were found unfavorable for sprouting.

It was also shown that low oxygen environment brought about elimination of apical dominance of the buds in the apex eyes at the base or stem end. On the basis of anatomical evidence it was suggested that in freshly harvested potato the skin is more permeable to oxygen and thus it is more susceptible to inhibitory effects of oxygen. Upon aging the periderm becomes thicker and less permeable to oxygen.

Though in many seeds dormancy is due to a lack of oxygen as a consequence of impermeability of seed coat to oxygen, Meyer and
Anderson (1956) mention that the seeds of *Typha latifolia* germinate better under low oxygen pressure.

It was concluded by Goddard (1939) that the lack of the enzyme carboxylase was the metabolic block that was overcome when dormancy of ascospores of *Neurospora* was broken by heat treatment. Sussman *et al* (1956) found that up to two hours heat treatment caused production of large amount of increase of acetaldehyde and ethanol. After two hours activated cells no longer produced these fermentation products. Fluoroacetate and cysteine poisoned germination and respiration but only after two hours of incubation subsequent to activation. These data together with the data on Krebs cycle acids were taken to indicate that activation induces the origin of glycolytic system which may work through Krebs cycle.

**Possible Relationship between the Respiratory Process and Chemical Changes in Germination**

Palmer (1959) found that dominancy of nutgrass tubers in a chain was less under high oxygen levels and higher as the oxygen concentration was lowered. In view of the role auxin played in apical dominance he interpreted the results as indicating that higher oxidative destruction of auxin decreased the effective auxin concentration which was needed by the tubers in order to exert dominancy. He further found a higher peroxidase activity in germinated tubers compared to dormant or those in which germination was inhibited with amino triazole treatment. Goldarce (1951) showed that hydrogen peroxide-peroxidase system was essential for the enzymic oxidation of indoleacetic acid. Galston and Siegel (1954) obtained evidence suggesting
that high oxygen tension results in peroxide formation which causes the peroxidative destruction of indoleacetic acid. Gerschman, et al (1954) suggested a probable mechanism of peroxide formation under high oxygen tension. They expected that high oxygen tension would increase the formation of oxidizing free radicals (OH*, HO2*) finally leading to formation of H2O2.

Paleg and Gordon (1956) showed that the observed increase in indoleacetic acid content by addition of certain polyphenols was not due to inhibition of indoleacetic acid oxidase but resulted from an enzymatically enhanced phenolic oxidation of tryptophane to indoleacetic acid. In a more recent paper (Gordon and Paleg, 1961) they presented evidence that the sequence

\[
\text{Phenols} \xrightarrow{\text{O}_2} \text{Quinones} \xrightarrow{\text{Phenolase}} \text{Quinone + Tryptophane} \xrightarrow{} \text{Indolepyruvic acid} \xrightarrow{} \text{Indoleacetic acid}
\]

occurred in plant material. The reaction was inhibited by replacement of air with nitrogen.

In contrast to above findings Briggs and Ray (1956) found that polyphenol oxidase could cause the inactivation of indoleacetic acid \textit{in vitro} provided catechol or pyrogallol were present. They interpreted their result as indicating occurrence of a reaction between indoleacetic acid and one of the intermediate compounds formed during oxidation of catechol or pyrogallol. \textit{In vitro} studies with a polyphenol oxidase and three phenols (catechol, chlorogenic acid and caffeic acid) by Leopold and Plummer (1961) supported Briggs and Ray's view as all the three phenols oxidatively formed quinone - indoleacetic acid complexes.
These findings thus indicate that levels of indoleacetic acid can probably be influenced by the respiratory process in more than one way. Evidence is also available which points out that indoleacetic acid may itself influence the respiratory process and its intermediary substrates.

Nance and Cunningham (1950) suggest that indoleacetic acid may influence the partition of pyruvate and related products of glycolysis into various metabolic ways. Further studies (Nance, 1958) demonstrated that indoleacetic acid inhibits the incorporation of radioactivity from acetate-1-C\(^{14}\) into pectic substances, hemicellulose and lipids but enhances incorporation into organic acid and carbon dioxide.

Commoner and Thimann (1941) concluded that the effectiveness of auxin as a growth hormone is closely related to its effect on respiratory process and that four-carbon acid respiratory system represents the link in this relationship. Marre and Forti (1958) observed that during the first period following the indoleacetic acid treatment of isolated pea internode segments high energy phosphate level increases while the level of phosphate acceptor decreases and suggested that enhancement of oxygen uptake by indoleacetic acid may be a consequence of activation of some oxidative system rather than a mere consequence of the stimulation of growth. Further studies (Marre et al. 1960) suggested that the activation of overall oxidative metabolism cannot be taken as a necessary link in the main chain of reaction through which auxin stimulates growth.

Tonzig and Trezzi (1950) obtained evidence that ascorbic acid oxidation may also be concerned in the mechanism of action of auxin.
On the basis of evidence from various workers Tonzig and Marre (1956) formulated the hypothesis that many of the physiological effects of auxin could be determined by its capacity to hinder ascorbic acid oxidation in the tissue and thus to maintain a low concentration of dehydroascorbic acid. Among the various possible mechanisms through which the effects of auxin on ascorbic acid oxidation could be transmitted to metabolic functions of fundamental importance, one of the most probably was suggested by the close association of ascorbic acid-dehydroascorbic acid to the reduced-oxidized glutathione system through widely occurring dehydroascorbic acid reductase (Yamaguchi and Joslyn, 1951). It was thought that an auxin induced decrease of ascorbic acid oxidation rate should produce a shift towards the reduced form of glutathione which would affect the reduced state of sulfhydryl groups and thus the biochemical behavior of several enzymes and cofactors (Racker, 1954). Further study of the proposed mechanism by Marre and Arrigoni (1957) led them to suggest that the auxin induced increase of reduced glutathione may be due either to an increased rate of reduction of glutathione by the TPN linked dehydrogenase and glutathione system or to a decreased rate of oxidation of reduced glutathione by some system or compound capable of accepting electrons from this substrate. Among these the dehydroascorbic acid was considered to be most important. They argued that since ascorbic acid oxidation is perhaps the only reaction reproducibly influenced in vitro by auxin at physiological concentrations and ascorbic acid/dehydroascorbic acid ratio is significantly shifted in vivo towards reduction by auxin treatment; therefore glutathione reduction could easily be achieved by auxin through inhibition of ascorbic acid oxidation, with a consequent lowering of dehydroascorbic
acid level and finally the decrease of reduced glutathione oxidation rate. Dehydrogenase activation would then be carried on by the highly reduced glutathione system.

Another role for the ascorbic acid oxidase system in control of the respiratory process has been suggested by Lieberman and Biale (1956). It was earlier reported by Akazawa and Uritani (1954) that chlorogenic acid, a natural phenolic substance in sweet potato, uncoupled oxidation from phosphorylation during oxidation of Krebs cycle intermediates. Lieberman and Biale (1956) found that catechol in concentrations of $10^{-4}$ M and higher almost completely inhibited both oxidation and phosphorylation. When ascorbic acid was added to the complete \( 	ext{-ketoglutaric system containing catechol, the inhibitory effect was prevented. Substitution by chlorogenic acid for catechol in those experiments gave identical results. From these data it was concluded that the oxidized phenols or their polymerization products strongly inhibited oxidative phosphorylation and that when polyphenols were kept in the reduced state by ascorbic acid they were ineffective in uncoupling phosphorylation.}
METHODS AND MATERIALS

Source of Plant Material

**Sugarcane (Saccharum officinarum)**

Variety C.P. 36-105, grown on the Louisiana State University Experiment Station farm was used in all laboratory, greenhouse and field experiments, with the exception of one laboratory test.

Apparently healthy sugarcane stalks were selected and used for the treatment the same day or were kept overnight, without stripping, with cut ends dipped in water, and used the following day. Some entire cane stalks were also buried in soil at the end of the season for use in laboratory and greenhouse experiments in winter. When required the entire cane stalks were dug out, washed clean with water, and then prepared for treatment in the usual way.

**Nutgrass (Cyperus rotundus)**

Nutgrass tubers were transplanted into freshly filled pots and within a few months an abundance of tubers had developed. These tubers were used in all the studies.

Preparation of Plant Material for Treatment

**Sugarcane**

The top immature section of the stalk was discarded and the remainder of the stalk was stripped clean of the sheath so that all the buds were exposed completely.
Nutgrass

Several pots were emptied, the soil removed with water, and single tubers or chains of 5 tubers connected by a rhizome were separated from the tuber system. All roots growing on the tubers were removed by clipping with scissors and blotting the tubers with paper towels. Extraneous moisture on the tubers was also removed by this process.

Preparation of Gas Mixtures

The treatments consisted of 0, 20, 60, and 100 per cent oxygen. Nitrogen was used as an inert gas. Thus 0 per cent oxygen contained nitrogen only and the 20 per cent oxygen treatment contained 80 per cent nitrogen. A 10 per cent oxygen treatment was also used in the Warburg studies. The gas mixtures were prepared in tanks and a separate tank was used for each mixture. To insure uniform discharge of gas for the duration of the experiment the tanks were fitted with pressure regulators which discharged the gas at a uniform pressure of 5 pounds per square inch.

Methods of Treatment

Sugarcane

Whole stalks were treated in 7.5 foot long aluminum cylinders made from 10 inch diameter aluminum pipes. One end of the cylinder was closed by an aluminum disk having a gas inlet cock. The other end could be closed by a removable aluminum disk containing a gas outlet cock. This disk was bolted on after the stalks had been placed
in the cylinder, thus making the cylinder air tight when the inlet and outlet cocks were closed. In order to saturate the gas with moisture it was bubbled through water before it entered the cylinder. A piece of tygon tubing was attached to the outlet cock and was kept dipped in a beaker of water. This device thus insured that the whole system was sealed from the outside atmosphere and was at atmospheric pressure.

After proper sealing the air within the cylinder was replaced by flushing with about 200 liters of the appropriate gas. All through the duration of treatment this gas was continuously passed through the cylinder at the rate of 150 ml per minute.

**Nutgrass**

Both single tubers and chains of five tubers were treated with the gas in 125 Erlenmeyer flasks. After preparation the tubers were placed in the flasks which were closed immediately. Each flask contained 50 single tubers or 3 chains of 5 tubers each. Flasks containing tubers being treated with the same gas were connected to each other by tubing. As in case of sugarcane the gas was bubbled through water before entering the first flask and was also made to pass out into the atmosphere through water.

Prior to treatment, the flasks were flushed by a rapid stream of appropriate gas for 5 minutes and thereafter a continuous stream of the appropriate gas was passed through the flask all through the duration of treatment at the rate of 50 ml per minute.
Germination Studies

Sugarcane

Germination of sugarcane buds after treatment was studied under (a) field conditions, (b) greenhouse conditions, and (c) laboratory conditions.

Types of planting materials used in different experiments were as described below:

(1) Full length cane: The entire stalks were planted after treatment.

(2) Half length cane: The stalks after treatment were cut from the middle thus dividing them into upper and lower half portions. Both portions were nearly equal in length but since the upper portion of a sugarcane stalk has shorter internodes the upper half of the stalk contained more buds.

(3) Single bud cuttings: The stalk was first divided into upper half and lower half, as described above, and then into single bud cuttings having only one bud on each cutting. The single bud cutting from upper half and lower half were planted separately.

(4) Isolated buds: In the laboratory experiments single buds attached to a minimum of stem tissue were used. The amount of tissue attached to each bud was an average of 2.0 to 2.5 cm long, 0.75 to 1.0 cm wide and 0.5 to 0.75 cm thick.

Only firm and uninjured buds were used for these studies. When full length and half length canes were used the visually injured buds were removed before planting and the number of firm uninjured buds was recorded for calculation of germination percentage. In the cases of single bud cuttings and isolated buds the injured buds were discarded.
(a) **Field Experiments:**

Because only enough stalks for planting one main plot could be treated in a cylinder at one time the planting of experiments was spread over 13 days in 1960-61 experiment and 10 days in 1961-62 season.

**Field Experiment 1960-61.** The experiment was conducted in a silt loam soil located at the Essen Lane Farm of the Louisiana State University Experiment Station. A randomized split plot design was used with the following treatments and three replications.

(I) Duration of treatments: (a) 24 hours, (b) 48 hours

(II) Oxygen concentrations: (a) 0%, (b) 20%, (c) 60%, (d) 100%

The main plot carried the duration treatments. On each main plot there were subplots carrying four oxygen concentration treatments.

Each subplot consisted of two 40 foot rows 40 inches apart. Full length stalks (12 stalks per treatment) were planted end to end and were covered immediately with 5 inches of soil. The ridges were not shaved in the spring.

**Field Experiment 1961-62.** The experiment was planted in a silt loam soil at the sugarcane farm of Louisiana State University Experiment Station. A split-split-plot design was used with the following treatments and three replications.

(I) Duration of treatment: (a) 24 hours, (b) 48 hours

(II) Type of planting material: (a) Upper half of the stalk

(b) Lower half of the stalk

(III) Oxygen concentrations: (a) 0%, (b) 20%, (c) 60%, (d) 100%

The main plots carried the duration treatments. On each main plot there were 2 subplots with two types of planting material. Each
subplot was divided into 4 sub-sub-plots carrying 4 oxygen concentra-
tion treatments.

The subplot consisted of one 35-foot row. All cultural condi-
tions were same as in 1960-61 experiment.

(b) Greenhouse Experiments:

Greenhouse experiments were conducted with single bud cuttings, half length cane, and full length cane. In all experiments 5 stalks were planted in one treatment.

The single bud cuttings were planted in metal flats (1.5 foot x 1.0 foot x 0.25 foot) with the buds on the upper side and were covered with one inch layer of soil. Buds from one stalk were planted in a single flat with the buds from upper and lower part of the stalk in separate rows. The flats were grouped into replications; one stalk from each treatment comprising one replication. The flats within a replication were randomized.

Half length canes were planted in wooden flats (7.0 foot x 2.5 foot x 0.5 foot) filled with soil. When possible the stalks were planted with buds located laterally, but in some cases the curvature of the stalk made it unfeasible. Stalks were covered with 1.5 inch layer of soil. The depth was, however, not uniform due to curvature in some stalks.

(c) Laboratory Experiments:

Buds were removed from the treated stalks (5 stalks per treat-
ment) as described under "sugarcane" and immediately placed in dis-
tilled water. The buds were then dipped in 4 to 6 per cent sodium hypochlorite solution for 3 minutes. In the experiment with variety
Co. 290 the outer bud scales were removed prior to dipping in sodium hypochlorite solution to reduce infection of buds by microorganisms lodged below the scales. After sterilization the buds were rinsed in sterile distilled water. These buds were then transferred aseptically to culture vessels.

An agar medium described by Johnson and Buchholtz (1961) for culture of rhizomes of Agropyron repens was prepared in Pyrex beakers using 0.8 per cent Difco agar and 100 ml portions were poured in 400 ml beakers. The beakers were then covered with aluminum foil wrapped tightly around the rim. The beakers were then sterilized for 30 minutes at 15 pounds per square inch pressure.

After transfer of the buds the aluminum foil was replaced and pressed tightly all along the rim except near the spout where it was raised slightly in order to allow the vapors of sodium hypochlorite to escape. The vapors were allowed to escape for 12 hours in the first experiment and for 3 hours in subsequent ones. In order to reduce the possibility of contamination during these periods the beakers were kept in a dark room, separated from the laboratory by double doors, which reduced chances of contamination during the period of exposure. After this period the beakers were removed to growth rooms and kept at 30-32°C.

**Nutgrass**

Tubers of nutgrass were germinated on filter paper in 9 cm diameter petri plates with covers. The tubers were covered with another filter paper.

The tubers and filter papers were kept moist at all times with distilled water. Temperatures during the germination period varied
between 28-30°C.

Respiratory Studies

Preparation of Tissue for Respiratory Measurements

(a) Sugarcane (preparation of buds):

Immediately after treatment the buds, along with a small amount of nodal tissue, were removed from nodes by a spatula. The nodal tissue as well as the outer bud scales were removed and the bud quartered longitudinally with a sharp knife. Quartered slices from five stalks were mixed and a representative sample was drawn for each flask. Each flask received 200 mg of tissue, 3 ml of a buffer solution of pH 6.0 which was 0.05 M and 0.02 M with regard to potassium phosphate and sucrose, respectively.

(b) Nutgrass (preparation of homogenate):

Homogenates for oxidation studies were prepared according to the methods described by Palmer (1959). Five grams of the tubers to be homogenized were sliced 3-4 mm thick and the slices were placed in an omni-mixer containing 50 ml of a 0.05 M potassium phosphate buffer of pH 6.0. The grinding vessel was placed in an ice water bath and the tissue was homogenized for two minutes. The crude homogenate was filtered through two layers of cheese cloth to eliminate crude fractions. The filtrate was centrifuged at 500 x g for 10 minutes at 2°C. The resulting supernatant was used for respiratory studies.

Determination of Oxygen Uptake and Carbon Dioxide

Oxygen uptake and carbon dioxide evolution of whole tissue and of homogenates were determined by using a Warburg respirameter and
conventional methods described by Umbreit et al (1957). All experiments were conducted at 25°C. Gas exchange is expressed as microliters per hour per gram of fresh weight in case of whole tissue and as microliters per hour per milligram of nitrogen where homogenate was used.

Double arm flasks were used for determining oxygen uptake in experiments involving respiration under different oxygen concentrations. Appropriate gas or gas mixture was continuously flushed through each flask during the period of equilibration. At the end of equilibration stop cocks of the manometers and flasks were closed and gas exchange determined.

**Ascorbic Acid Oxidation:**

A 2 ml aliquot of a homogenate from treated tissue was placed in the main compartment of the Warburg flask. The ascorbate was prepared in buffer and adjusted to pH 6.0 with NaOH. A 0.5 ml quantity of ascorbate was tipped from the side arm following equilibration. The final concentration of ascorbate was .048 M. Gas exchange was measured for 2 hours.

**Nitrogen Determination**

Protein nitrogen in homogenates was determined by the modified method of Hiller et al (1948).

**Digestion**

A grain of Cu SeO₄ was placed in the Kjeldahl flask followed by 8 ml of digestion mixture (500 ml of H₂O + 75 g of Na₂SO₄ + 500 ml concentrated H₂SO₄) and three ml of homogenate. The samples were digested with moderate heat until 5 minutes after clearing, allowed
to cool, and 20 ml of distilled water was then added to the flask.

Distillation

Ten ml of 2 per cent boric acid (2 g/98 ml) was placed in the receptor flask to trap the ammonia and two drops of methyl red (indicator) was added for titration purposes. Bubbles were started in the distilling flask and then, the digestion mixture was added through the funnel and followed with 15 ml of NaOH solution (200 g NaOH + 300 ml water). The boric acid flask was immediately raised to obtain surface bubbles in the solution. The bubbles were maintained for one minute after the trap solution became colorless after which the receptor flask was lowered about one cm below the outlet for 30 seconds for draining before removing the sample.

Titration

The sample was titrated back to the original color with dilute sulfuric acid of known acid factor. The ml of acid required times the acid factor gave the mg of nitrogen in the sample.

Spectrophotometry

Preparation of Extract

The same procedure as described under respiratory studies was used with the exception that only 25 ml of buffer at pH 7.0 was used. The supernatant was turbid and therefore it was diluted 20 times. Even after dilution it was not completely clear. In order to check that the curve obtained might be due to scattering by the suspended particles, the supernatant was further centrifuged at 6000 X g in the ultra centrifuge for 30 minutes. The curve obtained from this clear
recentrifuged supernatant was similar to the one obtained from the first supernatant but the absorption was reduced to a very great extent. Since recentrifuging brought about only a quantitative and not a qualitative difference in the curve it was decided to use the supernatant after the first centrifugation.

**Measurements**

The supernatant was examined for its absorption spectrum on the Model DU Beckman spectrophotometer. The same buffer in which the tissue was homogenized was used as the standard representing zero absorbance. Preliminary examination of the absorption spectrum, covering wavelengths from 250 μm to 600 μm were made. The observations in these examinations were taken at 20 μm intervals. Where peaks or troughs in the absorption spectrum were indicated, in preliminary examination, absorption at shorter intervals, down to 1 μm, were taken.

**Paper Chromatography**

The supernatant prepared for spectrophotometry was also used, without dilution, for paper chromatography.

A solvent system consisting of isopropanol-water (8:2, v/v) was used. Ehrlich reagent (1.0 g. p-dimethylaminobenzaldehyde in 100.0 ml of 1.0 N hydrochloric acid) was used for detecting phenolic compounds.
RESULTS

PART I. SUGARCANE

Germination Studies

Germination studies were carried out under three different environmental conditions (field, greenhouse and laboratory) and with four types of planting material (full length cane, half length cane, single bud cuttings and isolated buds) and therefore the germination recorded in different experiments was not comparable in all respects. For example, in case of half length cane planted in flats a bud emerged from the soil surface only after it had grown one and a half inches or more compared to one inch in the case of a single bud cutting. Further, the buds in half length cane were in the "side" position or in some cases in "down" position compared to the favourable "up" position for germination in single bud cuttings. Thus the actual germination pattern, during the initial phases of germination, was recorded more accurately in the case of single bud cuttings. Differences in the case of other types of plantings have been pointed out at the appropriate place in describing results.

Preliminary studies under greenhouse conditions showed that more buds germinated during the early germination phase with 0 per cent oxygen treatment when compared to 100 per cent oxygen treatment. The final germination was, however, always higher with 100 per cent treatment. This germination pattern with 0 and 100 per cent treatments
was obtained consistently in experiments with full length cane and single bud cuttings. The germination curves with 20 per cent and 60 per cent treatments on the other hand did not show any definite pattern when compared to each other or with that of 0 per cent or 100 per cent treatments. Therefore in presentation of results the main emphasis has been given to comparison of 0 per cent and 100 per cent treatment effects and in graphical representation the curves for 60 per cent treatment effects have been omitted. The curves for 20 per cent treatment effects have been included as representing response to normal atmospheric conditions.

Germination Under Greenhouse Conditions

Single Bud Cuttings

Results obtained with single bud cuttings are shown in Figures 1 to 4. Germination percentages calculated for whole stalks for 24 hour treatments (Fig. 1) show that first germination was recorded three days after planting after 0 per cent oxygen treatment and five days after planting with the 100 per cent treatment. All buds germinated within 10 days of planting, the germination period thus consisted of 8 days only. The buds treated with 100 per cent oxygen after a slow start germinated rapidly and the curve for 100 per cent treatment crossed the 0 per cent curve about 3 days after start of germination. In the next two days the germination in 100 per cent treatment was almost complete while in 0 per cent treatment it was still under progress, though at a much slower rate, and reached near its maximum level six days after start of germination.

The germination curves for upper and lower stalks (Fig. 2) were
essentially similar to the one described above for whole stalk. There were, however, differences in the extent and nature of response to the 0 and 100 per cent treatments. In buds from the upper half the difference between 0 and 100 per cent treatments was narrower than in case of buds from lower half of the stalk. Further the 0 per cent treated buds from the upper half reached their maximum germination values much later than the corresponding buds from lower half. Thus a sort of delaying influence on germination of buds from the lower half by 0 per cent oxygen treatment was indicated.

In the case of 48 hours treatment the nature of germination curves (Fig. 3 and 4) computed for the whole stalk as well as for upper and lower halves were similar to those described for 24 hours duration treatment. The slow rate of germination with 100 per cent oxygen treatment was, however, more clearly indicated here. The delayed germination of buds from the upper half with 0 per cent treatment was also evident.

Comparisons of final germination percentage values obtained under 24 hour treatments and 48 hour treatments are presented in Table 1 below.

Table 1. Comparison of final germination percentages in single bud cuttings obtained with 24 and 48 hour duration treatments.

<table>
<thead>
<tr>
<th>Stalk Part</th>
<th>0 Per Cent Oxygen</th>
<th>100 Per Cent Oxygen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper Buds</td>
<td>48 Hours Treatment</td>
<td>24 Hours Treatment</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>86.2</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>92.0</td>
</tr>
<tr>
<td>Lower Buds</td>
<td>72.7</td>
<td>65.0</td>
</tr>
<tr>
<td></td>
<td>86.4</td>
<td>86.9</td>
</tr>
<tr>
<td>Whole Stalks</td>
<td>87.7</td>
<td>77.5</td>
</tr>
<tr>
<td></td>
<td>93.5</td>
<td>89.6</td>
</tr>
</tbody>
</table>

In general the data show an increase in germination percentage
Figure 1. The influence of treatment with different oxygen concentration for 24 hours on germination of single bud sugarcane cuttings. Germination percentages computed for the whole stalk.
Figure 2. The influence of treatment with different oxygen concentrations for 24 hours on germination of single bud sugarcane cuttings. (A) Upper stalk (B) Lower stalk.
due to an increase in length of treatment. The increase was, however more marked in case of 0 per cent treatment. The buds from the lower portion were benefited less with 100 per cent treatment and actually showed a slight decrease in germination with 48 hours treatment.

**Half Length Cane**

The results obtained with half length cane are presented in Figures 5 to 8.

Germination percentage calculated for whole stalks for the 24 hours treatment (Figure 5) show that the first germinating bud emerged from the soil 9 days after planting. The germination phase was nearly over in 8 days. Both rate and extent of germination were lower throughout the germination period in the case of buds treated with 0 per cent oxygen when compared with 100 per cent oxygen treated buds. Thus the characteristic stimulation of germination in the early stage with 0 per cent treatment, exhibited in the case of single bud cuttings, was absent.

The germination curves obtained for buds treated for 48 hours (Fig. 7) were similar to those exhibited with 24 hours duration treatments except that the final germination percentage of lower buds was higher with 0 per cent treatment.

A comparison of final germination percentages obtained with 24 hours and 48 hours treatment is presented in Table 2. Values obtained in a similar comparison using single bud cuttings have also been included in the table.
Table 2. Comparisons of final germination percentages in half length stalks with 24 hours and 48 hours duration treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stalk part</th>
<th>0 Per Cent Oxygen</th>
<th>100 Per Cent Oxygen</th>
<th>Difference obtained with single bud cutting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>48 hours treatment (A)</td>
<td>24 hours treatment (B)</td>
<td>Difference (A-B)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Upper Stalk 69.5</td>
<td>52.6</td>
<td>+16.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower Stalk 40.0</td>
<td>33.3</td>
<td>+ 6.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Whole Stalk 54.1</td>
<td>41.3</td>
<td>+12.8</td>
</tr>
</tbody>
</table>
Figure 3. The influence of treatment with different oxygen concentrations for 48 hours on germination of single bud sugarcane cuttings. Germination percentage computed for whole stalk.
Figure 4. The influence of treatment with different oxygen concentrations for 48 hours on germination of single bud sugarcane cuttings. (A) Upper stalk. (B) Lower stalk.
Figure 5. The influence of treatment with different oxygen concentrations for 24 hours on germination of half lengths of sugarcane stalk. Germination percentage computed for whole stalk.
Figure 6. The influence of treatment with different oxygen concentrations for 24 hours on germination of half lengths of sugarcane stalk.

(A) Upper stalk. (B) Lower stalk.
Figure 7. The influence of treatment with different oxygen concentrations for 48 hours on germination of half lengths of sugarcane stalk. Germination percentage computed for whole stalk.
Figure 8. The influence of treatment with different oxygen concentrations for 48 hours on germination of half lengths of sugarcane stalk.
(A) Upper stalk. (B) Lower stalk.
The data show that the differences obtained with single bud cuttings and half length cuttings are of identical nature. The effects of long duration treatments, however, have been brought out more clearly when compared to those obtained with single bud cuttings. The adverse effects of 100 per cent treatment for 48 hours duration were more evident, as in this case the germination decreased by 9.4 per cent compared to a decrease of 0.5 per cent in case of corresponding treatment with single bud cuttings.

Table 3 shows the differences in germination percentages between single bud cuttings and half length cane with different treatments.

Table 3. Effect of dividing the stalk into single bud cuttings on germination

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 Per Cent</th>
<th>100 Per Cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Single (A)</td>
<td>Half (B)</td>
</tr>
<tr>
<td>Stalk Part</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper</td>
<td>86.2</td>
<td>52.6</td>
</tr>
<tr>
<td>Lower</td>
<td>65.0</td>
<td>33.3</td>
</tr>
<tr>
<td>Whole Stalk</td>
<td>77.5</td>
<td>41.3</td>
</tr>
</tbody>
</table>

24 Hours Duration

Upper          | 100.0     | 69.5         | +30.5           | 100.0      | 73.1     | +26.9          |
Lower          | 72.7      | 40.0         | +32.7           | 86.4       | 36.0     | +50.4          |
Whole Stalk     | 87.7      | 54.1         | +33.6           | 93.5       | 54.9     | +38.6          |

A comparison of differences between single bud cuttings and half length cane under 0 per cent oxygen treatment shows that the factor which caused an increase in germination percentage when the stalk
was cut into single bud cuttings influenced both the upper and lower buds identically. Further, since these differences were more or less the same under both durations of treatment the increase in germination was probably the result of dividing the stalk into single bud cuttings, thereby removing the inhibition caused by apical dominance. Thus the effect of separating the buds from each other, or in other words the removal of apical dominance, may be estimated to be in between 33.6 per cent and 36.2 per cent, which are the values (computed on the basis of whole stalk) by which the germination increased due to separation of buds from each other.

When compared with 0 per cent oxygen treatment the differences in germination between single bud cuttings and half length cane under 100 per cent oxygen treatment were wider in the case of lower buds and were appreciably influenced by the longer treatment. It may be noted that the widening of the differences was due to smaller degree of stimulation of germination of lower buds of half length cane with 24 hours treatment and actual inhibition in the case of longer duration treatment.

In the case of upper buds treated with 100 per cent oxygen the difference between germination of single bud cuttings and half length cane was narrow when compared with corresponding differences under 0 per cent treatment. This narrowing of differences could be due to the fact that effects of apical dominance were only removed to the extent of 27 per cent by dividing the 100 per cent oxygen treated stalk into single bud cuttings. As the single bud cuttings under 100 per cent oxygen treatment showed very high germination (92% and 100%) this explanation does seem to be valid. Therefore it can be assumed that in the upper buds of half length cane the 100 per cent oxygen treatment partially
removed apical dominance.

**Full Length Cane**

The effects on full length cane of 48 hours treatment with different oxygen concentrations are shown in Figure 9. The cane in this experiment was germinated in sand and is thus not strictly comparable to other experiments discussed earlier. However, the superiority of 0 per cent oxygen treatment over 100 per cent oxygen treatment has been clearly brought out. Thus the response of full length cane, as indicated by this experiment, appears to be different from that exhibited by single bud cuttings or half length cane.

**Field Experiments**

Tables 4 and 5 show the results obtained in field experiments in 1960-61 and 1961-62 seasons respectively. The data were statistically analyzed. Since the variations were in terms of percentages the data were transformed to corresponding Arcsin values for the purpose of calculations (Snedecor 1956 pp. 316-320). The average values were also computed by Arcsin method and then transformed to percentages.

Besides the presence of soil heterogeneity and fluctuations in environmental conditions, the field experiment differed from green house experiments in the following two respects:

1. The stalks under field condition were covered with a 5 inch layer of soil compared to a one and a half inch layer in green house experiments. This means that under field conditions there was a greater time lag between the actual germination of the buds and the recording of germination. Thus the germination recorded under field conditions
Figure 9. The influence of treatment with different oxygen concentrations for 48 hours on germination of full length sugarcane stalk.
actually reflected the germination capacity plus the growth rate of germinated bud for the 5 inch distance through the soil.

2. Since the experiments were planted in fall the buds which might have germinated in fall were subjected to low soil temperatures during winter, leading to mortality in some cases. The low temperatures particularly affected the 1961-62 experiments, the temperature having been below freezing for several days.

Field Experiment In 1960-61

The experiment was planted with full length stalks. The data in Table 5 show that germination in the 0 per cent oxygen plots of 24 hours duration was higher all through the observation period when compared to 100 per cent oxygen treatment. In the case of the 48 hours duration treatment, the 0 per cent oxygen treatment plots showed higher germination only between March 12 and March 30, however, the difference between the two treatments was rather low, being 0.9 per cent on March 12, 1.8 per cent on March 23, and 2.3 per cent on March 30. On two dates which 0 per cent oxygen treatment showed lower germination (March 7 and April 28) the differences between the two treatments were practically negligible, being 0.4 per cent and 0.6 per cent respectively. On the whole the data may be taken as showing a slight superiority of 0 per cent oxygen treatment over 100 per cent oxygen treatment.

Though none of the treatment effects or the interaction of the two were statistically significant, the data may be taken as indicating superiority of the 0 per cent oxygen treatment over the 100 per cent oxygen treatment under 24 hours duration and to a lesser extent under 48 hours duration.
Table 4. Germination percentage of sugarcane buds under field conditions as affected by pretreatment with different oxygen concentrations. (Season 1960-61)

<table>
<thead>
<tr>
<th>Date of Observation</th>
<th>Length of Treatment</th>
<th>Oxygen Concentrations</th>
<th>24 Hours</th>
<th>48 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0%</td>
<td>20%</td>
</tr>
<tr>
<td>3/7/61</td>
<td></td>
<td></td>
<td>22.1</td>
<td>12.2</td>
</tr>
<tr>
<td>3/12/61</td>
<td></td>
<td></td>
<td>26.0</td>
<td>15.2</td>
</tr>
<tr>
<td>3/23/61</td>
<td></td>
<td></td>
<td>30.1</td>
<td>23.0</td>
</tr>
<tr>
<td>3/30/61</td>
<td></td>
<td></td>
<td>33.2</td>
<td>25.9</td>
</tr>
<tr>
<td>4/28/61</td>
<td></td>
<td></td>
<td>40.3</td>
<td>32.0</td>
</tr>
</tbody>
</table>

The data also show that increasing the length of treatment with 100 per cent oxygen treatment increased germination while the reverse was true for 0 per cent treatment.

Field Experiment in 1961-62

The experiment was planted with half length cane. Germination data presented in Table 5 show that germination in this experiment was low compared to 1960-61 experiment. The data again were statistically not significant both with respect to treatment effects and their interactions. A comparison of germination percentages under 0 per cent and 100 per cent oxygen treatments, however, shows that buds from the lower half of the stalk treated with 0 per cent oxygen for 24 hours showed slightly better germination up to April 3. The same was true for buds from both upper and lower portions under 48 hours duration treatment. However, the data taken on April 18 show a definite superiority of 100
Table 5. Germination percentage of sugarcane buds under field conditions as affected by pretreatment with different oxygen concentrations. (Season 1961-62)

<table>
<thead>
<tr>
<th>Date of Observation</th>
<th>Length of Treatment</th>
<th>24 Hours</th>
<th>48 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stalk Part</td>
<td>0%</td>
<td>20%</td>
</tr>
<tr>
<td>2/25/62 Upper</td>
<td>12.5 13.9 16.2</td>
<td>14.8</td>
<td>12.3 14.4 12.3</td>
</tr>
<tr>
<td>Lower</td>
<td>15.5 13.7 11.8</td>
<td>12.1</td>
<td>13.2 12.7 14.4</td>
</tr>
<tr>
<td></td>
<td>Av. for Whole Stalk</td>
<td>14.0 13.8 14.0</td>
<td>13.4</td>
</tr>
<tr>
<td>3/4/62 Upper</td>
<td>13.6 14.8 16.8</td>
<td>15.9</td>
<td>13.3 16.0 13.1</td>
</tr>
<tr>
<td>Lower</td>
<td>15.5 15.1 12.1</td>
<td>15.3</td>
<td>14.5 15.1 14.2</td>
</tr>
<tr>
<td></td>
<td>Av. for Whole Stalk</td>
<td>14.0 14.9 14.4</td>
<td>15.6</td>
</tr>
<tr>
<td>3/18/62 Upper</td>
<td>14.5 17.3 18.3</td>
<td>16.9</td>
<td>14.9 17.5 14.9</td>
</tr>
<tr>
<td>Lower</td>
<td>16.9 15.6 15.1</td>
<td>15.6</td>
<td>15.4 15.1 16.2</td>
</tr>
<tr>
<td></td>
<td>Av. for Whole Stalk</td>
<td>15.7 16.4 16.7</td>
<td>16.2</td>
</tr>
<tr>
<td>4/3/62 Upper</td>
<td>18.9 21.9 23.4</td>
<td>20.5</td>
<td>22.0 23.2 19.8</td>
</tr>
<tr>
<td>Lower</td>
<td>22.3 19.8 21.5</td>
<td>21.3</td>
<td>21.3 20.3 18.3</td>
</tr>
<tr>
<td></td>
<td>Av. for Whole Stalk</td>
<td>20.6 20.8 22.4</td>
<td>20.9</td>
</tr>
<tr>
<td>4/18/62 Upper</td>
<td>25.8 30.8 29.2</td>
<td>30.1</td>
<td>28.1 32.1 30.0</td>
</tr>
<tr>
<td>Lower</td>
<td>25.1 23.4 27.6</td>
<td>25.8</td>
<td>28.2 28.7 26.2</td>
</tr>
<tr>
<td></td>
<td>Av. for Whole Stalk</td>
<td>25.4 27.1 28.4</td>
<td>27.9</td>
</tr>
</tbody>
</table>
per cent oxygen treatment over 0 per cent oxygen treatment in the case of buds both from lower and upper half under both durations of treatments.

Even though the data from field experiments were statistically not significant, when considered in conjunction with greenhouse experiments the following effects of 0 per cent and 100 per cent oxygen treatment seem to be indicated.

1. With full length cane the 0 per cent oxygen treatment is superior to 100 per cent oxygen treatment.
2. With half length cane or single bud cuttings the 100 per cent oxygen treatment gives better germination.
3. Increasing the length of treatment duration increases germination both under 0 per cent and 100 per cent oxygen treatments in half length cane.

Laboratory Experiments

Germination studies under laboratory conditions were conducted with only 24 hours duration treatments and two varieties of sugarcane. Two methods of recording germination were adopted. In the first experiment, using variety C.P. 36-105 the germination activity of buds was recorded under two categories as described below.

1. The partially germinated buds. This category included the buds in which the maximum growth attained by the bud did not exceed 0.5 cm after showing clearly distinguishable swelling as indicated by separation of ventrical side of the bud scales from the nodal surface or by separation of the two halves of the bud scales.
2. Fully germinated buds. All buds in which the growth of bud exceeded 0.5 cm were recorded under this category.
In spite of surface sterilization buds showed fungal growth, particularly on the bud scales. The percentage of buds showing fungal growth under different germination categories is shown in Table 6.

Table 6. Percentage of buds showing fungal growth

<table>
<thead>
<tr>
<th>Germination Stage of buds</th>
<th>Oxygen Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Per Cent</td>
</tr>
<tr>
<td>Ungerminated</td>
<td>13.6</td>
</tr>
<tr>
<td>Partially germinated</td>
<td>53.4</td>
</tr>
<tr>
<td>Germinated</td>
<td>15.9</td>
</tr>
<tr>
<td>Total</td>
<td>82.9</td>
</tr>
</tbody>
</table>

The data show that more germinated buds had fungal growth in 100 per cent oxygen treatment. None of the organisms found are parasitic organisms of sugarcane.

The effect on germination of the oxygen treatments has been shown in Figure 10A. The curves clearly show that the total germination (partial + full) was high in case of 0 per cent oxygen treated buds up to three days after treatment when compared with 100 per cent oxygen treatment. However, on the sixth day germination was higher in 100 per cent oxygen treated buds and it continued to rise till it reached 88.6 per cent on the tenth day. No increase in germination was recorded in the 0 per cent oxygen treated buds between the third and sixth days. After the sixth day the curve for 0 per cent oxygen treatment rose until it reached 77.6 per cent level on the tenth day. The curves were thus essentially identical with those obtained with single bud cuttings. However, a point worth considering here is that the stimulation in germination in initial stages with single bud cuttings
represents buds with a growth of at least one inch. The same part of the curve in this experiment was a count of partially germinated buds, none of which had developed more than 0.5 cm at the time of observation. This seems to indicate that complete expression of the effects of 0 per cent oxygen is dependent on the bulk of the tissue to which the bud is attached since the stem tissue attached to an isolated bud was very little compared to the stem tissue attached to a bud in a single but cutting. An alternate explanation can be put forward on the basis of difference in per cent buds with fungal growth in these two treatments. The data in Table 6 shows that even though a higher per cent of germinated buds had fungal growth in 100 per cent oxygen treatment more of them developed into fully germinated buds. It is therefore possible that the partially germinated buds were not vigorous enough to develop in the presence of fungal growth and hence most of them remained in that stage.

Figure 10B shows the curves for fully germinated buds. The lower rate and extent of development of partially germinated buds in 0 per cent treatment when compared to 20 and 100 per cent treatments is clearly evident.

The experiment was repeated with variety Co. 290. Since bud scales were removed in this case it was not possible to detect the swelling of buds at early stage. Therefore only those buds which had clearly made some growth could be counted as partially germinated buds.

The removal of outer buds scales resulted in reducing fungal growth to a very large extent and it was confined to only few buds.

The curves for fully germinated buds were more or less identical with curves obtained with variety C.P. 36-105.
Figure 10. Influence of treatment with different oxygen concentrations for 24 hours on germination of isolated buds of sugarcane.  
(A) Total germination (partial + fully germinated)  
(B) Fully germinated only.
In both experiments a comparison of final germination percentages indicated that the capacity of buds to develop after partial germination seem to be dependent on oxygen percentage of the treatment. This appears evident when the data computed in terms of relative bud activity are examined in Table 7. It shows that treatment with 100 per cent oxygen increases the capacity of development of partially germinated buds by 1.5 times over 20 per cent oxygen treatment and 2 to 3 times over 0 per cent oxygen treatments. These results seem to be significant in view of the fact that nearly identical values for relative bud activities were obtained in spite of difference in variety, method of observation and near absence of fungal growth in experiments with variety Co. 290.

Table 7. Relation between partially and fully germinated sugarcane buds as affected by pretreatment with various oxygen concentration.

<table>
<thead>
<tr>
<th>Treatment (per cent oxygen concentration)</th>
<th>Per cent fully germinated (A)</th>
<th>Per cent partially germinated (B)</th>
<th>Bud Activity A</th>
<th>Relative bud activity (taking values for 100% treatment as 1.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variety C.P. 36-105</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>40.0</td>
<td>48.1</td>
<td>0.419</td>
<td>1.0</td>
</tr>
<tr>
<td>20</td>
<td>20.1</td>
<td>52.9</td>
<td>0.280</td>
<td>0.668</td>
</tr>
<tr>
<td>0</td>
<td>11.3</td>
<td>65.9</td>
<td>0.147</td>
<td>0.350</td>
</tr>
<tr>
<td>Variety Co. 290</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>95.4</td>
<td>4.6</td>
<td>0.954</td>
<td>1.00</td>
</tr>
<tr>
<td>20</td>
<td>51.7</td>
<td>31.0</td>
<td>0.632</td>
<td>0.662</td>
</tr>
<tr>
<td>0</td>
<td>34.3</td>
<td>40.0</td>
<td>0.461</td>
<td>0.483</td>
</tr>
</tbody>
</table>
Respiratory Studies

Values for oxygen uptake and respiratory quotient of buds measured under normal atmospheric conditions after various oxygen treatments are shown in Tables 8 and 9. In general oxygen uptake was greater in upper buds and increased with the increase in duration of treatment. The oxygen uptake in 20, 60 and 100 per cent oxygen treated buds did not vary significantly among themselves when compared to that in 0 per cent oxygen treated buds. Further, the variations among the three oxygen concentration treatments did not follow any particular pattern while the 0 per cent oxygen treated buds consistently showed a lower oxygen uptake compared to these treatments. In general the oxygen uptake in the 0 per cent oxygen treatment was six tenths of the uptake measured for 100 per cent treatments. This ratio was slightly higher for lower buds under both durations of treatments and was in general lower for 48 hours treatments. The lowering of the ratio in the latter case was to a great extent due to a higher oxygen uptake by the 0 per cent oxygen treated buds.

As regards respiratory quotient the values for 0 per cent oxygen treated buds were higher and except for upper buds under 48 hours duration were all indicative of organic acid metabolism. The lower buds in general showed higher R.Q. values. The difference between respiratory quotients in 0 per cent and 100 per cent oxygen treatment was also higher in lower buds. However, it decreased with duration of treatment, the narrowing of the difference being largely due to reduction in respiratory quotient in 0 per cent oxygen treatments.

Respiration of sugarcane buds in different oxygen concentrations was also measured. The data presented in Table 9 show that oxygen
uptake increased with increase of oxygen concentration under which the buds were respiring. In all cases upper buds showed higher oxygen uptake.

Table 8. Respiration of sugarcane buds (oxygen uptake in ul/g, fresh weight) measured immediately after treatment with different oxygen concentrations.

<table>
<thead>
<tr>
<th>Treatments (Per cent Oxygen Concentration)</th>
<th>Duration of Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 Hours</td>
</tr>
<tr>
<td></td>
<td>Upper Buds</td>
</tr>
<tr>
<td>0.0</td>
<td>125.2</td>
</tr>
<tr>
<td>20.0</td>
<td>244.5</td>
</tr>
<tr>
<td>60.0</td>
<td>187.0</td>
</tr>
<tr>
<td>100.0</td>
<td>210.9</td>
</tr>
<tr>
<td></td>
<td>48 Hours</td>
</tr>
<tr>
<td></td>
<td>Upper Buds</td>
</tr>
<tr>
<td>0.0</td>
<td>154.9</td>
</tr>
<tr>
<td>20.0</td>
<td>233.6</td>
</tr>
<tr>
<td>60.0</td>
<td>272.6</td>
</tr>
<tr>
<td>100.0</td>
<td>255.6</td>
</tr>
</tbody>
</table>

Table 9. Respiratory quotient of sugarcane buds measured immediately after treatment with different oxygen concentration.

<table>
<thead>
<tr>
<th>Treatment (Per cent Oxygen Concentration)</th>
<th>Duration of Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 Hours</td>
</tr>
<tr>
<td></td>
<td>Upper Buds</td>
</tr>
<tr>
<td>0.0</td>
<td>1.80</td>
</tr>
<tr>
<td>20.0</td>
<td>1.08</td>
</tr>
<tr>
<td>60.0</td>
<td>0.88</td>
</tr>
<tr>
<td>100.0</td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td>48 Hours</td>
</tr>
<tr>
<td></td>
<td>Upper Buds</td>
</tr>
<tr>
<td>0.0</td>
<td>1.19</td>
</tr>
<tr>
<td>20.0</td>
<td>1.08</td>
</tr>
<tr>
<td>60.0</td>
<td>1.12</td>
</tr>
<tr>
<td>100.0</td>
<td>1.11</td>
</tr>
</tbody>
</table>
Table 10. Respiration of sugarcane buds (oxygen uptake in ul/g. fresh weight) under different oxygen concentrations.

<table>
<thead>
<tr>
<th>Per Cent Oxygen Concentration</th>
<th>Upper Buds</th>
<th>Lower Buds</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.0</td>
<td>131.7</td>
<td>122.3</td>
</tr>
<tr>
<td>20.0</td>
<td>333.5</td>
<td>300.3</td>
</tr>
<tr>
<td>60.0</td>
<td>451.7</td>
<td>410.2</td>
</tr>
<tr>
<td>100.0</td>
<td>487.5</td>
<td>453.3</td>
</tr>
</tbody>
</table>
DISCUSSION

It has already been pointed out that while effects of 0 per cent and 100 per cent oxygen treatments on germination of sugarcane buds showed a definite pattern under all conditions, those obtained with 20 per cent and 60 per cent oxygen treatments were not consistent. The inconsistency in response with 20 and 60 per cent oxygen treatments and the consistent nature of the germination pattern obtained with 0 and 100 per cent oxygen treatments, irrespective of type of planting material used or environmental factors, may be interpreted as showing that the factors through which 0 and 100 per cent oxygen treatment affected germination process were more basic in nature and therefore not influenced by environmental or other factors. Any deviation from these two oxygen concentrations (0 per cent and 100 per cent) probably diluted their respective effects and made the germination process susceptible to change in environmental conditions or fluctuations in other factors within the stalk itself, thus accounting for the erratic response of 20 and 60 per cent treated buds.

The possibility that the influence of 0 per cent oxygen treatment on germination may be dependent on the amount of stem tissue attached to the bud has already been pointed out in comparing the response of isolated buds with that of single bud cuttings.

In view of the findings of Bieleski (1958) that cane stored for 48 hours in anaerobic conditions showed very high alcohol production it can be visualized that the treatment of stalks with 0 per cent oxygen will result in production of considerable amounts of partially degraded products of glycolysis. On return to normal atmospheric conditions
these products would be available for immediate utilization by buds for their development. The greater the bulk of the tissue per bud the larger will be the amount of these partially degraded products of glycolysis. The amount of tissue per bud in cases of isolated buds was probably just enough to activate the buds only up to partially germinated stage while in cases of single bud cuttings it was enough to push their development beyond that stage.

In cases of stalks treated with 100 per cent oxygen these partially degraded products would be at a very low level partly because of their rapid utilization and also due to suppression of glycolysis. It would be only after return to normal atmospheric conditions that the rate of glycolysis would reach the required levels.

Sussaman et al (1956) noted in case of ascospores of *Neurospora* that a rapid increase in glycolysis, resulting in formation of ethanol and acetaldehyde, was the first change, after activation, in the sequence of events leading to germination. If a higher glycolytic rate is also the first event in the sequence leading to the germination process in sugarcane then it is obvious that while the stalks are under 100 per cent oxygen atmosphere a higher glycolytic rate will not be attained. In that case the germination process will only start after the cane is removed to normal atmospheric conditions.

An alternate explanation has also been considered which attributes the difference between response of isolated bud and single bud cuttings to the effect of fungal growth present in isolated buds. It was considered that the partially germinated buds in 0 per cent treatment might not have developed due to fungal growth and hence initial stimulation with this treatment was not expressed in terms of
fully germinated buds. While there is a certain degree of probability of this explanation being true the results obtained with full length cane tend to support the argument based on difference in the bulk of tissue attached to buds. Thus in the case of full length cane, where the amount of tissue per germinated buds was considerably high, the 0 per cent treatment gave higher germination throughout. This may be attributed to higher amount of degradation products of glycolysis available to each bud.

That no beneficial effect of 0 per cent treatment was obtained in case of half length cane can perhaps be attributed to the fact that in half length canes there are two more cut ends in each stalk. The buds near the cut ends are in an unfavorable position so far as germination is concerned because of rotting of the stem tissue near the cut ends after planting. A percentage of these buds would thus be deprived of the benefit of the changes brought about by 0 per cent treatment and therefore the stalk would fail to exhibit the stimulatory effect of 0 per cent oxygen treatment. It may also be noted that in the case of half length cane the germinated buds emerged from the soil 6 days later than the single bud cuttings. Thus the buds in single bud cuttings, by their earlier emergence from the soil, escaped the adverse effects of rotting of stem tissue even though there were cut surfaces on each side of every bud.

The influence of 100 per cent oxygen concentration was indicated more clearly in experiments with isolated buds because the effect of 0 per cent oxygen treatment was considerably reduced due to reduction in bulk of the stem tissue to a near minimum level. The results showed that the activation of buds, as indicated by
swelling of buds, was not influenced by 100 per cent treatment. On the other hand, development of buds as indicated by values of relative bud activity, seem to be primarily dependent on oxygen concentrations.

The comparison of data for half length cane and single bud cuttings indicated that 100 per cent oxygen treatment may produce some sort of inhibitory effect on germination. It was noted that in contrast to 0 per cent treatment the increase in duration of 100 per cent treatment did not increase germination of the lower buds. In fact it decreased the germination by 9 per cent in half length cane. However, this adverse effect was annulled by dividing the stalk into single bud cuttings after treatment.

In the case of upper buds on the other hand, the 100 per cent treatment seems to have benefited germination in a similar way to dividing the stalk into single bud cuttings.

If the increase in germination due to dividing the stalk into single bud cuttings is considered as removal of top dominance then it may be suggested that effects of 100 per cent oxygen treatments are similar and additive to those associated with top dominance in the case of lower buds, while in the case of upper buds they seem to remove top dominance to a certain extent.

From these results it can therefore be concluded that the effect of 100 per cent treatment on germination is conditioned by some other factor. Since the effect of 0 per cent treatment on upper and lower buds was similar, though varying in degree, it is apparent that this "other factor" is not the one affected by 0 per cent.

The oxygen uptake data recorded for upper and lower buds do not seem to provide any clue as to the nature of the interaction between
effects of 100 per cent treatment and the hypothetical factor.

In summary it may be said that in so far as the effect of different oxygen concentrations on germination of sugarcane buds is concerned there are three basic factors involved. One of the factors stimulates germination when the stalks are treated by 0 per cent oxygen and the other when the canes are treated with 100 per cent oxygen. The third factor seems to interact with effects of 100 per cent treatment in the presence of conditions producing top dominance.
PART II. NUTGRASS

Germination Studies

Germination studies using nutgrass tubers were undertaken to supplement those made with sugarcane. The oxygen concentrations used were the same as in the case of sugarcane but the duration of treatments was extended up to 144 hours. A nutgrass tuber has a number of buds and many of them may germinate. In the experiments a tuber was considered to have germinated when it showed a clear emergence of any one bud, whether or not that bud made further growth.

As in the case of sugarcane it was noted that responses of nutgrass tubers to 20 and 60 per cent oxygen treatments were not consistent, and hence emphasis in consideration of results was again confined to responses obtained with 0 and 100 per cent oxygen treatments.

Preliminary studies were conducted with the single tubers as well as with chains of 5 tubers each. These studies suggested that the moisture on the tubers as well as the moisture percentage of gas or gas mixtures during treatment should not be very high. When the moisture on the tubers was high or the moisture percentage of gases was brought to near saturation levels by passing them through a loosely packed column of wet cheese cloth before these entered the treatment flasks, then a large number of tubers under 20, 60, and 100 per cent oxygen treatments germinated during the treatment period itself. Thus the germination data recorded after treatment under high moisture
conditions partly represented the effect of these gas concentrations during germination rather than the effect of pre-treatment. In case of 0 per cent oxygen treatment, on the other hand, the germination data reflected entirely the effect of pre-treatment. In view of this, in later experiments the moisture on the tubers was completely dried out by keeping them in open air for some time before placing them in flasks for treatment. Further, the gases were bubbled through only one inch of water in a 125 ml Erlenmeyer flask to keep a lower moisture percentage in the gases. It was not found possible to control the moisture precisely and probably this fluctuation in moisture content caused some variation between different experiments.

In the first set of experiments the comparative response to treatments with different oxygen concentrations was studied. Results obtained with 24 and 48 hour duration treatments (Figs. 11 and 12) exhibit a distinct stimulatory effect of 100 per cent oxygen treatment during the early phase of germination. The germination of tubers treated with 0 per cent oxygen on the other hand was low during the early germination phase. However, it later rose and either reached almost the same level as attained by tubers treated with 100 per cent oxygen (Fig. 11B) or was superior to that (Fig. 11A and 12A, 12B). The germination curves further show that the stimulatory effect of 100 per cent treatment decreased with increase in length of treatment. These differences in response to 0 and 100 per cent oxygen treatments were discernible in the case of single tubers as well as with tubers in chains and were reflected in final germination percentages. Thus in case of single tubers under 24 hours duration treatment, the final germination of 0 per cent oxygen treated tubers was only 2 per cent
Figure 11. Influence of treatment with different oxygen concentrations for 24 hours on germination of nutgrass tubers.
(A) Single tubers. (B) Tubers in chain of five.
Figure 12. Influence of treatment with different oxygen concentrations for 48 hours on germination of nutgrass tubers. (A) Single tubers. (B) Tubers in chain of five.
higher than that of 100 per cent oxygen treated tuber; whereas, under 48 hours duration treatment there was a difference of 12 per cent. Similarly, in the case of tubers in chains, the germination of 0 per cent oxygen treated tubers was 4 per cent less than that of 100 per cent oxygen treated tubers under 24 hours treatment, but it was 4 per cent more under 48 hours treatment.

The curves in Figures 11 and 12 also show that compared to single tubers the tubers in chains were more responsive to stimulatory effects of 100 per cent oxygen treatment indicating that the presence of apical dominance influences the effects of 100 per cent oxygen treatment.

When single tubers and tubers in chains were treated for 144 hours the germination in 0 per cent oxygen treated tubers was higher throughout the germination phase when compared to germination in 100 per cent oxygen treated tubers.

A second experiment was run in which the single tubers were treated from 24 to 120 hours. The results are graphically presented for 24, 48, 72 and 120 hours duration treatments (Figs. 13 and 14). The curves for 96 hours duration treatment were more or less similar to 72 hours duration treatment. An inspection of the curves shows that the 100 per cent oxygen treated tubers showed better germination over the whole germination phase with 24 hours treatment while with the 72 and 120 hours duration treatments the germination of tubers treated with 0 per cent oxygen was consistently superior to that of 100 per cent treated tubers. The response to 48 hours duration treatments was intermediate between the responses to 24 hours and 72 hours duration treatments. The findings in this experiment thus also support
Figure 13. Influence of treatment with different oxygen concentrations on germination of single tubers of nutgrass. (A) 24 hours treatment. (B) 48 hours treatment.
Figure 14. Influence of treatment with different oxygen concentrations on germination of single tubers of nutgrass. 
(A) 72 hours treatment. (B) 120 hours treatment.
the conclusions drawn from earlier experiments that the germination of tubers is benefited by increase in duration of 0 per cent oxygen treatment while the reverse was true for 100 per cent oxygen treatment.

That these stimulatory and inhibitory effects may become diluted when the tubers after treatment are returned to normal atmospheric conditions is shown by the curves in which the germination percentage on a particular day after treatment was plotted against the duration of treatment (Fig. 15).

The curves indicate an inhibitory effect of 100 per cent treatment when the length of treatment was extended beyond 48 hours. This effect became apparent 1 day after treatment and disappeared between 2.5 days and 3 days. The stimulatory effect of 0 per cent oxygen treatment was obtained with increase in length of treatment up to 96 hours. Treating tubers beyond this duration with 0 per cent oxygen decreased germination when compared to the other shorter durations. The stimulatory effect of 0 per cent oxygen treatment was apparent two days after treatment and disappeared between the fourth and fifth day after germination.

The results obtained with both the experiments can be summarized as below:

1. The stimulatory effect of the 100 per cent oxygen treatment on germination was only expressed with the treatments of shorter duration and extending the length of treatment beyond this limit caused inhibition. The stimulatory effects of the 0 per cent treatment on the other hand required a longer duration of treatment for their expression.

2. The stimulatory or inhibitory effects of 100 per cent oxygen treatments were discernible earlier (within one day after treatment)
Figure 15. Influence of length of oxygen treatments on germination of nutgrass tubers on different days after treatment. Germination on (A) one day, (B) two days, (C) two and half days, (D) three days, (E) four days, and (F) Five days. The points shown in black were obtained by interpolation.
and were relatively less stable, disappearing between 2.5 days and 3 days after treatment. In the case of 0 per cent treatment the effect on germination was not clearly evident before 2 days but was more stable than the effect of 100 per cent treatment.

3. As in the case of sugarcane, the effects of 100 per cent treatment seemed to be influenced in the presence of apical dominance, though not in the same ways. The 100 per cent treatment caused more stimulation of germination under the influence of apical dominance.

It was also shown in these experiments that with 48 hours duration treatment the stimulatory effects of both 0 per cent and 100 per cent oxygen treatments were expressed, and hence this treatment duration was selected for study of certain changes in treated tubers insofar as these were related to germination.

Spectrophotometric and Chromatographic Study of Extracts from Treated Tubers

Leopold and Plummer (1961) showed that indoleacetic acid forms addition products with quinones produced by the action of polyphenol oxidase. They presented spectrophotometric as well as chromatographic evidence for the formation of such complexes. Since Palmer (1959) demonstrated a high polyphenol oxidase activity in nutgrass tubers it is possible that a prolonged 100 per cent oxygen treatment may result in higher concentration of quinones. These quinones may then form complexes with native auxin present in nutgrass tubers and may thus modify their germination pattern.

In view of the above mentioned consideration the absorption spectra of neutral extracts of nutgrass tubers treated with 0 and 100 per cent oxygen for 48 hours were examined in the spectrophotometer...
Immediately after treatment and also 5 and 9 days after treatment. All extracts showed an absorbance curve somewhat similar to the one obtained by Leopold and Plummer (1961) for indoleacetic acid - caffeic acid complex. Thus the curve obtained from the tuber extracts showed a trough near about 265 μm wavelength, a peak near 278 μm and then a sharp drop until 500 μm followed by plateau between 500 and 600 μm.

The neutral extract was also chromatographed in isopropanol-water (8:2, v/v) solvent. In some chromatograms sprayed with Ehlrich reagent a very faint yellow spot was observed. The Rf for this spot was .65 while that observed for indoleacetic acid-phenol complex by Leopold and Plummer was .55. Thus it cannot be said with certainty that the substance observed spectrophotometrically was of the same type as indoleacetic acid - caffeic acid complex found by Leopold and Plummer (1961).

The variation in concentration of the substance, as measured by absorption at 265 μm and 278 μm wavelengths showed (Table 11) that though it was higher in 0 per cent oxygen treated tubers at the end of 48 hours treatment, comparatively more of it was present in 100 per cent treated tubers on fifth and ninth day after treatment.

The data presented in Table 11 can be correlated with the germination curves for 0 and 100 per cent oxygen treated tubers, however, in view of the uncertainty about the nature of the compound it can not be said without doubt that the changes reflect a change in auxin-phenol complex.
Table 11. Relative absorbance at 265 and 278 μm wavelength by neutral extracts of nutgrass tubers treated with 0 and 100 per cent oxygen for 48 hours.

<table>
<thead>
<tr>
<th>Wavelength in μm</th>
<th>Days on Which Measured</th>
<th>0% oxygen</th>
<th>100% oxygen</th>
<th>0% oxygen</th>
<th>100% oxygen</th>
<th>0% oxygen</th>
<th>100% oxygen</th>
</tr>
</thead>
<tbody>
<tr>
<td>265</td>
<td>0 days after treatment</td>
<td>0.86</td>
<td>0.69</td>
<td>1.09</td>
<td>1.05</td>
<td>1.16</td>
<td>1.31</td>
</tr>
<tr>
<td>278</td>
<td>5 days after treatment</td>
<td>0.98</td>
<td>0.83</td>
<td>1.19</td>
<td>1.21</td>
<td>1.20</td>
<td>1.39</td>
</tr>
</tbody>
</table>

Ascorbic Acid Oxidation

The importance of ascorbic acid oxidation in relation to physiological effects of auxin was pointed out by Tonzig and Marre (1955) and its effect on phosphorylation by Lieberman and Biale (1956). It was therefore thought advisable to examine the effect of 0 per cent and 100 per cent oxygen treatments on ascorbic acid oxidation capacity of the nutgrass tubers. The ascorbic acid oxidation by homogenate from tubers treated for 48 hours is shown in the table below.

Table 12. Ascorbic acid oxidation by extracts of nutgrass tuber treated with different oxygen concentration.

<table>
<thead>
<tr>
<th>Percentage</th>
<th>Oxygen Uptake per Milligram of Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 days after treatment</td>
</tr>
<tr>
<td>0</td>
<td>ul</td>
</tr>
<tr>
<td>124.8</td>
<td>161.4</td>
</tr>
<tr>
<td>94.3</td>
<td>162.1</td>
</tr>
</tbody>
</table>
The data show a higher ascorbic acid oxidation in 0 per cent treated tubers immediately after completion of treatment and also 9 days after treatment. On the fifth day after treatment the ascorbic acid oxidation capacity of tubers in both oxygen treatments was almost the same; though in a few preliminary experiments the 0 per cent oxygen treated tubers showed slightly higher oxidation than tubers treated with 100 per cent oxygen.
DISCUSSION

Germination studies with nutgrass tubers brought out the following differences in response of single bud tubers to 0 per cent and 100 per cent oxygen treatments.

1. The increase in length of treatment beyond 48 hours produced inhibitory effects on germination in the case of 100 per cent oxygen treatment while in the case of 0 per cent oxygen treatment increase in length of the treatment up to 96 hours continued to stimulate germination.

2. The effects of 100 per cent oxygen treatment were evident within a short interval after treatment; while the effects of 0 per cent oxygen treatment required comparatively longer time for expression.

3. The effects of 100 per cent oxygen treatment disappeared more rapidly than those produced as a result of 0 per cent oxygen treatment. From this it may be inferred that the effects produced by 0 per cent oxygen treatment were more stable than the effects produced by 100 per cent oxygen treatment.

The longer duration of treatment required under 0 per cent oxygen to produce a stimulatory effect may be interpreted as indicating that the treatment involves a change in a large amount of some substance. This conclusion is also supported by the evidence obtained from germination studies with isolated buds of sugarcane.

In contrast it may be assumed that the effects of 100 per cent
oxygen treatment may be concerned with a change in a substance present only in comparatively small quantity. The possibility that the factor concerned in effects of 100 per cent oxygen treatment does not involve a large bulk of the tissue was considered in the case of sugarcane. Further the fact that the effects of 100 per cent treatment are influenced by the presence of apical dominance, both in case of sugarcane and nutgrass, indicates that the substance involved in the effects of 100 per cent oxygen treatment may be auxin or a substance of similar nature. Thus this interpretation also falls in line with the assumption that the effects of 100 per cent treatment are concerned with the change in a substance which is present in comparatively small quantity.

A review of the literature suggests that the 100 per cent oxygen treatment might affect the germination process in the following ways:

1. The auxin level may be changed by (a) peroxidative oxidation of auxin under 100 per cent oxygen treatment (Galston and Siegel, 1954) resulting in a lower auxin level; (b) formation of auxin or auxin precursor under 100 per cent oxygen treatment as a result of phenolic oxidation of tryptophane to indoleacetic acid (Gordon and Paleg, 1961); (c) lowering of auxin level by formation of auxin-phenol complex due to higher quinone formation under 100 per cent oxygen treatment (Leopold and Plummer, 1961).

2. The quinones formed under the influence of 100 per cent oxygen treatment may form compounds which uncouple oxidative phosphorylation (Lieberman and Biale, 1956) and thereby reducing the supply of energy needed for germination process.
3. A pure oxygen atmosphere may bring about higher ascorbic acid oxidation. The oxidized form of ascorbic acid in its turn might oxidize reduced glutathione (Yamaguchi and Joslyn, 1951) and thus start a chain of reactions affecting many processes. It has been suggested by Tonzig and Marre (1956) that many physiological effects of auxin could be determined by its capacity to hinder ascorbic acid oxidation.

It is evident from what has been discussed above that the 100 per cent oxygen treatment can increase or decrease the level of auxin depending on the type of metabolic mechanism existing in the tissue concerned. In view of this the mere fact that the tubers treated with 100 per cent oxygen had slightly lower ascorbic acid oxidation activity is not sufficient to interpret the germination results on the basis of one of the mechanisms discussed above, though it does point out the possibility that one of these may be involved.

The influence of apical dominance on the effects of 100 per cent oxygen treatment has already been pointed out. It was also noted in the case of both sugarcane and nutgrass that under the influence of apical dominance the effects of 100 per cent oxygen treatment were modified, though not in a similar manner, in every case. In the case of lower buds of sugarcane the effects of 100 per cent oxygen treatment seem to be similar to that produced by the apical dominance. In the upper buds of sugarcane 100 per cent oxygen treatment seem to have resulted in partial removal of apical dominance. In the case of nutgrass the tubers in chains were stimulated much more than the single tubers by the 100 per cent oxygen treatment. In view of these different effects it was suggested that the effects of the 100 per cent oxygen treatment were modified by some other
factor. If auxin level is the factor influenced by 100 per cent oxygen then it is possible that the other factor may be kinetin or kinetin-like substance, as Wickson and Thimann (1958) suggested that the normal phenomenon of apical dominance depends on an interaction between auxin and kinetin-like substance.
SUMMARY

Sugarcane stalks were treated with 0, 20, 60, and 100 per cent oxygen for 24 and 48 hours and the effect of these treatments on germination was studied in full length, half length, single bud cutting and isolated buds. Germination studies were conducted under field, greenhouse and laboratory conditions. Respiration of buds under treatment conditions and after treatment was also investigated. The germination studies in sugarcane were supplemented by studies with nutgrass tuber conducted under laboratory conditions.

The germination curves of 0 and 100 per cent oxygen treated sugarcane buds showed a consistent pattern while those of 20 and 60 per cent oxygen treatments were inconsistent when compared to each other, or with 0 or 100 per cent oxygen treatments.

In the greenhouse study of single bud cuttings the 0 per cent oxygen treated buds showed early germination compared to 100 per cent oxygen treated buds but the final germination was always higher with 100 per cent oxygen treatment. Increasing duration of treatment in both 0 and 100 per cent oxygen treatments in general increased the germination of single bud cuttings but the increase was more in the case of 0 per cent treated buds. The increase was less in lower buds under 0 per cent oxygen treatment and there was a slight decrease in germination of lower buds under 100 per cent oxygen treatment.

In half length cane the 100 per cent treated buds showed higher germination throughout the germination phase. Increasing the duration
of 100 per cent oxygen treatment increased the germination of upper buds only and decreased that of the lower buds.

Comparison of germination of single bud cuttings and half length cane indicated that in the presence of apical dominance the effects of 100 per cent oxygen treatment on lower buds were similar and additive to it but resulted in partial removal of apical dominance in upper buds.

In the case of full length cane the 0 per cent oxygen treatment when compared to 100 per cent treatment showed a superior germination all through the germination phase.

Germination data from field experiments, though statistically not significant, seemed to support the results obtained in greenhouse experiments with half length and full length canes.

Laboratory experiments with isolated buds indicated the presence of two different factors; one depending on the bulk of the stem tissue and influenced by 0 per cent oxygen treatment; and the other influenced by 100 per cent oxygen treatment and primarily concerned with further development of swollen buds.

Oxygen uptake by sugarcane buds was higher under higher oxygen concentrations when compared to other concentrations. Buds treated with 0 per cent oxygen showed lower respiratory activity and higher R.Q. than under normal atmospheric conditions.

In nutgrass tubers the stimulatory effect of 100 per cent oxygen treatment on germination was only expressed with the treatments of shorter duration and extending the length of treatment beyond this limit caused inhibition. The stimulatory effects of the 0 per cent treatment required a longer duration of treatment for their expression.
The stimulatory or inhibitory effects of 100 per cent oxygen treatments were discernible earlier and were relatively less stable than the effects of 0 per cent oxygen treatment.

The effects of 100 per cent treatment seemed to be influenced by the presence of apical dominance.

In spectrophotometric studies the neutral extracts of tubers showed an absorbance curve similar to the one for caffeic acid-indoleacetic acid complex but the identity of the compound could not be confirmed chromatographically.

An overall consideration of the investigations pointed out that the 0 and 100 per cent oxygen treatments influence the germination process through different factors; that the factor influenced by 0 per cent factor is quantitatively larger; and the factor influenced by 100 per cent oxygen treatment is very much smaller in quantity and may be an auxin or auxin-like substance. The possibility of auxin-kinetin interaction was also considered.
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BIOGRAPHY

Narendra Singh Negi was born December 22, 1929 at village Moli, Utter Pradesh, India. He was graduated from Government High School, Lansdowne, in June, 1945.

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Major Field: Botany

Title of Thesis: Effects of Treatment with Different Concentrations of Oxygen on Germination of Sugarcane (Saccharum officinarum, L.) and Nutgrass (Cyperus rotundus, L.)

Approved:

[Signatures of examiners]

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures of other committee members]

Date of Examination:

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