AN ABSTRACT OF THE THESIS OF

Onesimus B. Mmolawa for the degree of Master of Science in Crop Science presented on December 12, 1986.

Title: GERMINATION AND DORMANCY OF MEADOWFOAM SEED

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Meadowfoam (Limnanthes spp.) is a potential oil seed crop being developed for commercial production in the Willamette Valley. The seeds are characterized by poor germination at warm temperatures. It would be desirable to overcome this temperature-related dormancy problem so that better stand establishment could be realized from early fall planting.

The objectives of this study were to determine the factors influencing seed germination of meadowfoam, characterize the types of dormancy involved, and develop methods of overcoming dormancy.

Experiments were carried out with different-aged seed lots of L. alba 'Mermaid,' L. floccosa Howell and a cross of L. floccosa x L. alba. Germination requirements were investigated by studying seed responses to temperature, light, oxygen, growth regulators, scarification, and chemical inhibitors.

The most favorable germination temperatures were 10, 5-15 and 10-20°C. No germination occurred above 20°C. Prechilling 5 days at 5°C promoted germination at higher temperatures. Potassium and thiourea effectively promoted germination, while kinetin, gibberellic acid and ethephon did not. Daily 8-h exposures to light inhibited germination, particularly at the warmer temperatures. Increasing the length of the daily light periods resulted in corresponding decreases in germination. Removal of portions of the seed coat improved germination, as did germination at higher concentrations of oxygen. The seed coat was not a barrier to water uptake by dormant seeds. Water-soluble extracts from the seeds inhibited germination of non-dormant seeds.

A number of treatments imposed on meadowfoam seed overcame dormancy partially or fully, suggesting that there are several mechanisms regulating seed dormancy. Evidence from this study suggests that the seed coat is partially responsible, acting as a barrier to diffusion of oxygen and because it is a source of germination inhibitors. Embryo dormancy is also implicated, in that seeds responded to treatment with potassium nitrate. For routine germination testing of meadowfoam, it is recommended that seeds be planted on a substrate moistened with 1 g L^{-1} KNO_3 and placed in the dark at 10°C for

14 days. If circumstances warrant, additional germination of dormant seed lots can be obtained by germination on substrate of KNO_3 in an atmosphere of 100% O_2.

GERMINATION AND DORMANCY OF MEADOWFOAM SEED

by

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DEDICATED TO:

My wife Constance Seipone Mmolawa and my deceased mother Choda Gabaswediwe Mmolawa and father Herbert Mphitang Mmolawa.

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INTRODUCTION

Meadowfoam (*Limnanthes* spp. family *Limnanthaceae*) is a potential oil seed crop being developed for commercial production in the Willamette Valley (Jolliff, 1981). The seeds are characterized by poor germination at warmer temperatures. As a result of this thermodormancy, seeding in the Willamette Valley is delayed until mid-October to obtain cool soil temperatures. Late fall seeding, however, has risks, since fall rains sometimes delay seeding and cool weather reduces growth of seedlings and hinders stand establishment. If the temperature-related dormancy could be overcome, better stand establishment could be realized from early plantings.

Various methods known to break seed dormancy in other species were investigated to determine possible requirements for germination of meadowfoam. These special treatments include: alternating temperature, prechilling, oxygen, light, mechanical scarification, potassium nitrate and other growth regulators and leaching of chemical inhibitors.

It is felt that better understanding of the processes involved in seed germination and dormancy of meadowfoam may lead to means of alleviating the dormancy problem.

The objectives of this study were to: (1) determine the factors influencing seed germination of meadowfoam, (2) characterize the types of dormancy involved, and (3) develop methods of overcoming dormancy.

The experimental results are presented in the form of a manuscript.

LITERATURE REVIEW

The behavior of meadowfoam (Limnanthes spp.) as it relates to seed germination has not been extensively studied. The only information available on seed germination is the contribution on temperature (Toy and Willingham, 1966; Miller et al., 1964), light and temperature (Cole, 1974) and secondary dormancy (Toy and Willingham, 1967). Other seed species with similar germination characteristics will be reviewed to try to understand the germination of meadowfoam.

Temperature

Temperature has a broad effect on the germination of seeds. Different seeds have different temperature ranges within which they germinate. Early work on seed germination responses was concerned very largely with the effects of temperature, and with ways of expressing the germination character of species of crop plants in relation to their behavior at different temperatures (Thompson, 1970). Sachs, who first studied the effects of constant temperature, proposed the use of three cardinal points to describe the germination character using the maximum and minimum temperatures outside of which germination cannot take place, and the optimum temperature to describe the intermediate temperature at which best

germination is attained in the shortest time (Koller, 1972). According to Toole (1973) temperature affects the total number of seeds germinating in response to the phytochrome reaction, because it affects the rate of rehydration and synthesis of phytochrome and rates of P_{fr} reversion, destruction and action. When some seeds are held at high temperature for a long time they become thermodormant. This dormancy induced by high temperature can be overcome by special treatments before seeds will germinate at lower temperature (Vidaver and Hsiao, 1975). This damaging effect of high temperature may be the result of changes in the properties of membranes and enzymes and protein denaturation (Hendricks and Taylorson, 1976, 1979; Christiansen, 1978; Levitt, 1969). Low temperature treatment is an essential prelude to germination in many seeds and high temperature may be inhibitory at the time of germination (Koller, 1972). Stokes (1952, 1953) associated the development at low temperature with evidence of enzymatic action indicated by increase of soluble nitrogen compound and an increased proportion of arginine among the amino acids. Winter germinators like Linum and Linaria require an optimum temperature of 12°C while Delphinium requires 25°C. Summer germinators need a very wide daily range of germination temperature (12 to 33°C) (Toole et al., 1955). Nemophila, Phacelia, and Phlox were found to be dark-germinators in

cool temperatures (21 to 24°C) and at 31°C germinate neither in light nor dark (Lehmann, 1912).

The use of daily alternations of temperature has become well established after extensive contributions of Harrington (1923) and Morinaga (1926). Steinbauer and Grigsby (1957), who tested 85 species of crop and weed seeds, concluded that between 70 and 80% of the species tested showed some positive response to alternating temperature. Seeds of some species fail to germinate at constant temperatures and others may show improved germination when alternating temperatures are used. The requirement of alternating temperature for dormancy breaking and germination is complicated by the fact that other factors such as light, moisture, seed maturation and storage may interact to modify the response. Evenari (1965) has distinguished between species in which alternating temperatures promote germination in light or dark, those responding only in dark, and those in which germination is promoted in the light. Other authors have also found that alternating temperature can produce germination in the dark in seeds which are otherwise said to have a requirement for light, for example, Nicotiana (Toole et al., 1957) and Poa pratensis (Toole and Borthwick, 1971). The effect of temperature on germination is not independent of other factors, for example, Physalis franchett seeds germinate well in the

dark between 5 and 15°C but between 15 and 35°C they require light and the light requirement increases with the temperature (Vegis, 1964).

According to Cohen (1958), alternating temperature causes a change in the macromolecular structure of the components in the seed which in the original form prevents germination. The temperature at which different seeds germinate and the range within which they germinate is determined by the source of the seeds, genetic differences within a given species as well as age (Mayer and Poljakoff-Mayber, 1975).

Prechill

Seeds of many temperate-zone plants require a long period under moist conditions at low temperatures (0-5°C) for germination. This treatment enables the seeds to germinate when they later experience a more favorable temperature for growth. Seeds with a chilling requirement cannot germinate until after the winter has passed even though conditions in late autumn may be favorable (Black, 1970). Luckwill (1952) found that growth-promoting substances appeared in the embryos of after-ripened seeds just before germination started, but did not appear in seeds held in dry storage.

In Heracleum sphondylium seeds, it has been suggested that some substances from the embryo metabolism at low

temperature diffuse into the endosperm and bring about the breakdown of storage proteins into amino acids which are required for growth (Stokes, 1954). In imbibed cherry (Prunus avium) seeds, increases the respiratory capacity of the embryo (Wareing, 1965). In blackberry (Rubus sp.) seeds, the inhibiting substances were found to be highest in the endosperm, lower in the testa, and lowest in the embryos. The inhibitors disappeared during low temperature treatment and this disappearance of the inhibitor was correlated with the breaking of dormancy and the ability of the seed to germinate (Villiers and Wareing, 1960).

Wareing (1965) reported that prechilling did not reduce the inhibitor content in Fraxinus excelsior, but prechilled embryos were found to contain germination promoters, which stimulate germination of unchilled embryos. Prechilled seeds germinated in the presence of the inhibitor.

In some seeds that are not deeply dormant, Taylorson and Hendricks (1971) suggested that the effect of chilling is associated with the presence of P_{fr} in the seeds and its reduced rate of reversion of P_r at the lowered temperatures.

Light

Light may either inhibit or promote germination of dormant seeds. But not all light-sensitive seeds are promoted by white light. Some seeds, like *Nemophila*, *Nigella* and *Phacelia* are light-inhibited. This is apparently due to the far-red component of the white light (Black, 1970). Other species like bromelidad (*Acanthostachys strobilacea*), and those of the *Liliaceae* family are equally inhibited by white light. The influence of the presence or absence of light on germination of seeds has been shown to be the function of light quantity, duration of irradiation and the time of irradiation in relation to imbibition time (Evenari, 1965). Evenari and Neumann (1953) showed that when lettuce seeds were imbibed for 8 hours, they showed an increase in photosensitivity, but beyond 8 hours more light brought a decline in germination. In *Nigella*, Isikawa (1957) found that the irradiation by white light of 12 hours duration inhibited germination, but short time illumination stimulated germination.

The suppression of germination of *Phacelia tanacetifolia* by white light increased with intensity and the inhibition was proportional to the duration of illumination (Schulz and Klein, 1963). In *Limnanthes*, light is not required for germination, and germination in

some seeds was inhibited by long duration of light (Toy and Willingham, 1966; Cole, 1976). Exposure of Nemophila insignis to long daily photoperiods inhibits germination (Black and Wareing, 1959).

The seed coat as a barrier to gases

It has been known for a long time that seed coats have a profound influence on the ability of many seeds to germinate. Most of these seed coat effects have been attributed to the preservation of seed dormancy (Wareing and Saunders, 1971). The seed coat may regulate germination by establishing a permeability barrier and interfering with water uptake required for imbibition and subsequent radicle protrusion, gaseous exchange, particularly oxygen uptake required for respiration and the outward diffusion of endogenous germination inhibitors (Mayer and Shain, 1974).

The seed coat and other structures surrounding the embryo can be removed, punctured, cut, broken or chemically injured to allow many light-requiring and light-inhibited seeds to germinate (Evenari, 1965). Most authors agree that the seed coats interfere with the gas exchange necessary for normal germination, because the seed coats are generally permeable to water, but relatively impermeable to gases. There can be no doubt

that the seed coats deeply modify the nature of seed respiration (Mayer and Poljakoff-Mayber, 1975).

Respiratory gases as well as ethylene, which is concerned with different aspects of metabolism, pass through the seed coat, to and from the embryo. Many studies show reduced germination occurs as a result of restricted oxygen passage; these include the following: Xanthium (Crocker, 1906, Davis, 1930; Shull, 1911, 1914; Thornton, 1935), Avena fatua (Atwood, 1914; Hart and Berrie, 1966; Hay, 1962, 1967), Malus sylvestris L. (Visser, 1956), Phalaris (Vose, 1956), Sinapis arvensis L. (Edwards, 1968b, 1969). Supporting evidence has come from increased respiration rates following removal of coats or enrichment of oxygen supply, enhanced germination in high concentration of oxygen and direct estimates of coat permeability to gases. In Xanthium, early work indicated that removal of the seed coat reduced dormancy of the upper seed, and in sufficiently high concentration of oxygen the upper and lower intact seeds had similar germination (Ballard, 1973). Hay (1962, 1967) has shown that imbibed hulls around soaked Avena fatua seeds can restrict the oxygen supply to the embryo sufficiently to induce dormancy, which can later be relieved by applying high levels of oxygen. Edwards (1968b,c) has suggested other processes in the seed coats which could retard the inward diffusion of oxygen: (i) high respiratory activity

in the active zone such as aleurone layers, (ii) the oxidation of phenols and (iii) development of mucilage in influencing germination (Gutterman et al., 1967; Heydecker et al., 1971).

Consumption of oxygen by seed coat phenolics is also suggested as a factor limiting oxygen supply to embryos that are responsive to increases in oxygen pressure (Come and Tissaoui, 1973). Why is the diffusion coefficient of oxygen in the (wet seed coat) imbibed seeds so low? According to Bewley and Black (1982), two factors are involved or implicated: (i) the presence of mucilage in and around the seed coat and (ii) the consumption of oxygen by the coat itself, thus severely reducing the amount that passes through to the embryo. In sugar beet, the seed coat may function as an oxygen barrier due to oxygen consumption by the integuments (Ohmura and Howell, 1962). The seed coat of Charlock seed presents no resistance to water uptake yet offers a resistance to oxygen diffusion (Edwards, 1968c, 1969). Embryos with seed coat removed could germinate at low oxygen tensions. Edwards (1968b) attributed the effect of the seed coat to the establishment of low internal oxygen concentration that led to the production of growth inhibiting substances in the embryonic tissue. These substances accumulate within the seed coat and when they reach a critical concentration they prevent subsequent cell elongation.

According to Edwards, the seed coat therefore acts both as a diffusion barrier for oxygen and as a deposit of endogenous inhibitors. In later stages this oxygen diffusion barrier may also control germination by impeding the oxidation and subsequent destruction of the endogenous inhibitors (Vose, 1962). The seed coat may retard the leaching of germination inhibitors from the embryo (Roberts, 1969; Amen et al., 1970).

According to some authors, an inhibition of normal gas exchange may be an inhibition of oxygen entry into the seed or it may be of carbon dioxide exit from the seed into the atmosphere (Leggatt, 1948). Leggatt went as far as suggesting that the inability of the intact seeds to germinate could be explained in the first case by the lack of the necessary oxygen and in the second case by the presence of carbon dioxide which produces dormancy. Forward (1949) found that carbon dioxide brought about dormancy in oats and that the dormant condition of freshly harvested grains was caused by accumulation of carbon dioxide. This dormancy was broken by pricking of the seed coats or increases in concentration of oxygen to counteract the carbon dioxide effect. According to Bewley and Black (1982), there is very little evidence to support the hypothesis that links the dormancy-imposing action of the seed coat with the accumulation of carbon dioxide inside the seed coat. However, there are reports (Bewley

and Black, 1982) indicating that high concentrations of carbon dioxide (20%-40%) have been found to inhibit germination. The argument advanced by these authors is that if the retention of carbon dioxide within the seed coat is indeed to play any part in the imposition and maintenance of dormancy, the gas would have to accumulate to a high level.

Scarification

Scarification is achieved by either mechanical (physical) or chemical means, for example by rubbing against an abrasive surface or treating with strong mineral acid. Concentrated sulfuric acid has been used experimentally for many years with considerable success on many species (Hopkins, 1923; Povilatus, 1956; Jordan et al., 1982). Seeds of many species were made permeable by ethyl alcohol, field bindweed (Callihan, 1961), crown vetch (Brant et al., 1971). Mechanical scarification developed by Hughes (1915) utilizing abrasion, particularly by rough surface, is probably the most common commercial treatment for impermeable seeds. In Bidens pilosa L., loss of photosensitivity of seeds after scarification was reported (Felippe, 1978), whereas in Sinapis arvensis L. there was an increase of sensitivity to light after scarification (Hsiao, 1980). In Nemophila insignis, destruction of the seed coat

resulted in loss of light dependence (Chen, 1968). Barcucumber (Sicyos angulatus) resulted in increased water absorption and germination after mechanical scarification (Mann et al., 1980). In Avena fatua, Atwood (1914) concluded that germination could be increased by various seed coat-breaking methods.

Higher oxygen tensions

With the exception of seeds of certain hydrophates, seeds will not germinate without oxygen (Come and Tissaoui, 1973). The supply of oxygen to the embryo of the seed is often impeded by the enclosing seed integuments, seed coat, fruit wall or both and thus in many species seeds require higher oxygen tensions for germination to proceed (Takahashi, 1985). During early imbibition, respiration rates of rehydrated embryos may increase even though oxygen concentration is low (Come and Tissaoui, 1973). The stimulation effect of oxygen on germination has been reported for several species (Wareing and Foda, 1957; Durham and Wellington, 1961; Ikuma and Thimann, 1963; Popay and Roberts, 1970; Major and Roberts, 1968; Edwards, 1969; Chen, 1970; Frank and Larson, 1970). In many seeds, the availability of oxygen for seed germination is linked with dormancy and for example, dormant cereal seeds such as wild oats (Avena fatua) and barley (Hordeum vulgare) can be induced to germinate by

higher oxygen tensions (Crocker and Barton, 1953). In 1914, Atwood reported that oxygen could break the dormancy of wild oats. Breaking of seed dormancy in rice (Oryza sativa L.) has been reported by Roberts (1961) to be enhanced by higher oxygen tensions. Sachs et al. (1981) found that clay coated pepper seeds germinated faster in an environment of 100% oxygen. In Xanthium pennsylvanicum, the upper seed is much more dormant and requires minimum oxygen concentration of 30 to 60% while the lower seed requires 4% to 6% (Thornton, 1935).

A more specific role of oxygen includes prevention of inhibitor production (Edwards, 1969), enhancement of pentose phosphate pathway activity (Roberts and Smith, 1977), alternate respiratory pathway activity (Esashi et al., 1982), and regulation of the respiratory pathways during seed germination (Roberts, 1973).

High partial pressure of oxygen is also capable of forcing germination which is inhibited by unfavorable environmental conditions, for instance, for light inhibited seeds, Nemophila insignis, it has been reported that the presence of oxygen overcomes the light inhibition (Chen, 1968).

Germination inhibitors

Germination inhibitors are of very wide occurrence in all types of plant tissues and in seeds. The view that

dormancy in many seed species is due to germination inhibitors is based on the following observations: (i) inhibitors are found in embryos of many seed species possessing embryo dormancy, (ii) leaching out of the inhibitor is found to promote germination of isolated, dormant embryos and (iii) embryo dormancy can be induced by treating non-dormant embryos with known inhibitor (Bewley and Black, 1982). A number of species in which such inhibitors have been detected is considerable (Evenari, 1949). Germination inhibitors are frequently present in various parts of the fruit, including the pericarp, endosperm, seed coat (testa) and embryo.

The existence of germination inhibitors is usually detected by testing their effect on the germination of seeds, such as in wheat or cress (Lepidium sativum). Water extract from rice retards the germination and growth of rice and lettuce (Ovcharov, 1977). Ching and Foote (1961) also found water- and ethanol-soluble growth inhibitors in extracts of dormant wheat seeds and it was postulated that loss of dormancy was due to the oxidation of these inhibitors. In cultivated oats (Avena) dormancy of the intact seeds has been associated with the presence of a water-soluble inhibitor in the hulls (Elliot and Leopold, 1953). Barley and oats exhibit dormancy when freshly harvested, but removal of the hulls permits the germination of the isolated caryopses (Elliot

and Leopold, 1953). Black (1959) produced evidence to show that in Avena fatua the inhibitory effect of the hulls is not primarily due to these substances but to the fact that they prevent leaching of other inhibitors from the caryopsis.

Oxygen may be involved in the inactivation of an inhibitor in birch (Betula spp.) seeds. On work done on Betula pubescens and B. verrucosa, Black and Wareing (1959) suggested the following hypothesis. The seed coat impedes oxygen entry and also contains an inhibitor. Lack of oxygen itself does not prevent germination but lack of oxygen in the presence of an inhibitor does. Roberts (1969) has suggested that either the inhibitor increases the oxygen requirement of the embryo or that high oxygen concentrations are necessary to overcome the effect of the inhibition. Dormancy in Xanthium seeds involves the presence of two soluble inhibitors. In the upper seed, the seed coat acts as a barrier to leaching of the water soluble germination inhibitors from the embryo. Increased oxygen tensions accelerate the enzymic oxidation of these inhibitors to inactive forms (Porter and Wareing, 1974; Wareing and Foda, 1957; Wareing, 1963). In Avena fatua there is some evidence that oxidation reaction involved in loss of dormancy is the oxidation of germination inhibitors to inactive forms. A similar influence of inhibitors was observed in Acer pseudoplatanus L.

(Webb and Wareing, 1972) in which outward leaching was limited by the testa. The requirement of high oxygen tension for germination was apparently not due primarily to the presence of the seed coat, but to the presence of an inhibitor, which disappears in high concentration of oxygen (Wareing and Saunders, 1971).

Germination promoters

Two chemical substances that promote germination are widely used: nitrates and thiourea. Nitrates have been long known to stimulate germination and break dormancy in many species. Species responsive include the graminaceous seeds and dicotyledonous weed species (Roberts and Smith, 1977). Potassium nitrate (KNO_3) is effective as a promoter of germination of light-sensitive seeds.

It has been shown that KNO_3 replaced after-ripening treatments in three seeds which require a period of dry storage at room temperature, for example, oats (Stokes, 1965). KNO_3 is the most widely used chemical for promoting seed germination in routine germination testing. The Association of Official Seed Analysts and the International Seed Testing Association recommend solutions of 0.1 to 0.2% KNO_3 for germination tests of many species. Schwendiman and Shands (1943) reported that a 0.2% solution of KNO_3 was equally as effective as prechilling in overcoming delayed germination of

germination of freshly-harvested seeds of oats. The action of nitrates varies with the temperature of germination (Toole and Toole, 1939). Thiourea, a sulfur-nitrogen containing compound, may substitute for after-ripening treatments. It is known to be very effective in promoting germination of some light-requiring or temperature-inhibited seeds such as lettuce (Thompson and Kosar, 1939). In the light-inhibited seeds of Phacelia, thiourea inhibits germination slightly in the dark and enhances the germination inhibition brought by white light (Evenari, 1965).

Kinetin is reported to promote dark germination of lettuce seed in low concentrations, thus replacing light requirement (Miller, 1956; Skinner et al., 1956). The effect of kinetin on light-inhibited seeds of Phacelia is quite different. Kinetin depresses dark germination slightly. As long as the seeds are kept in light, it has no stimulating effect but promotes germination after seeds are transferred to darkness (Evenari, 1965).

Gibberellins accumulate in the seed in response to the environment stimulus that triggers dormancy loss (Ross, 1984). The production of alpha-amylase by barley aleurone is now well known (Paleg, 1960; Yomo, 1960). It is often implied that gibberellins induce germination via amylase production (Amen, 1968; Galston and Davies, 1969). Gibberellins release dormancy in seeds which require

stratification (Ross and Bradbeer, 1968, 1971). The application of gibberellic acid (GA) will stimulate the germination of certain light requiring seeds, e.g. lettuce and birch (Khan, 1960), Lepidium virginicum (Toole and Cathey, 1959). Germination of Phacelia tanacetifolia is inhibited by light. GA releases this inhibition (Chen, 1970). GA partially stimulates the germination of Cruciferae vegetable seeds (Nakamura et al., 1960). There have been reports indicating that GA stimulates ethylene production from seeds (Stewart and Freebairn, 1969; Ketring and Morgan, 1970).

It has been known since the 1920s that ethylene would promote germination in certain species, but intensive investigations were not carried out until the 1960s. Dormancy in seeds of many species is broken by ethylene, supplied as gas directly or by means of ethylene generating chemicals such as ethephon (Ketring, 1977). In non-dormant varieties and after-ripened dormant varieties of peanut (Arachis hypogaea), ethylene has been shown to stimulate germination (Ketring and Morgan, 1969). Early work on seed germination of cereals and peanuts has shown that dormant seeds evolved less ethylene than non-dormant ones and ethylene evolution preceded radicle emergence (Taylorson and Hendricks, 1977). Ethylene affects seed germination in dormant seeds by interacting with some membrane fraction in the endoplasmic reticulum and the

protein body membrane (Bengochea et al., 1980; Hall et al., 1982). Ethylene is used to stimulate germination of several weed species such as *Striga asiatica*, *S. lutea* (Eplee, 1975; Egley and Dale, 1970), *Amaranthus retroflexus*, *A. albus*, *Chenopodium album* and *Ambrosia artemisifolia* (Taylorson and Hendricks, 1979). High temperature thermodormancy in lettuce seed is overcome by treatment with ethylene (Burdett, 1972).

MANUSCRIPT

GERMINATION AND DORMANCY OF MEADOWFOAM SEED

ABSTRACT

Meadowfoam (*Limnanthes* spp.) is a potential oil seed crop being developed for commercial production in the Willamette Valley. The seeds are characterized by poor germination at warm temperatures. It would be desirable to overcome this temperature-related dormancy problem so that better stand establishment could be realized from early fall planting.

The objectives of this study were to determine the factors influencing seed germination of meadowfoam, characterize the types of dormancy involved, and develop methods of overcoming dormancy.

Experiments were carried out with different-aged seed lots of *L. alba* 'Mermaid,' *L. floccosa* Howell and a cross of *L. floccosa* x *L. alba*. Germination requirements were investigated by studying seed responses to temperature, light, oxygen, growth regulators, scarification, and chemical inhibitors.

The most favorable germination temperatures were 10, 5-15 and 10-20°C. No germination occurred above 20°C. Prechilling 5 days at 5°C promoted germination at higher temperatures. Potassium and thiourea effectively promoted germination, while kinetin, gibberellic acid and ethephon did not. Daily 8-h exposures to light inhibited germination, particularly at the warmer temperatures.

Increasing the length of the daily light periods resulted in corresponding decreases in germination. Removal of portions of the seed coat improved germination, as did germination at higher concentrations of oxygen. The seed coat was not a barrier to water uptake by dormant seeds. Water-soluble extracts from the seeds inhibited germination of non-dormant seeds.

A number of treatments imposed on meadowfoam seed overcame dormancy partially or fully, suggesting that there are several mechanisms regulating seed dormancy. Evidence from this study suggests that the seed coat is partially responsible, acting as a barrier to diffusion of oxygen and because it is a source of germination inhibitors. Embryo dormancy is also implicated, in that seeds responded to treatment with potassium nitrate. For routine germination testing of meadowfoam, it is recommended that seeds be planted on a substrate moistened with 1 g L^{-1} KNO_3 and placed in the dark at 10°C for 14 days. If circumstances warrant, additional germination of dormant seed lots can be obtained by germination on substrate of KNO_3 in an atmosphere of 100% O_2.

Additional index words: Limnanthes alba Benth., Limnanthes floccosa Howell, Oxygen, Scarification, Prechilling, Potassium nitrate, Kinetin, Inhibitors, Thiourea, Photoperiod, Light.

GERMINATION AND DORMANCY OF MEADOWFOAM SEED

INTRODUCTION

Meadowfoam (Limanthes spp.) is a potential oil seed crop being developed for commercial production in the Willamette Valley (Jolliff, 1981). The seeds are characterized by poor germination at warm temperatures. As a result of this thermodormancy, seeding in the Willamette Valley is delayed until mid-October to obtain cool soil temperatures. Late fall seeding, however, has risks, since fall rains sometimes delay seeding and cold weather reduces growth of seedlings and hinders stand establishment. If the temperature-related dormancy could be overcome, better stand establishment could be realized from early plantings.

Dormancy in meadowfoam, as in many winter annuals, is an adaptive phenomenon allowing seeds to survive during adverse conditions and germinate when conditions are favorable (Nikolaeva, 1977; Stokes, 1965). Since meadowfoam is still in the early stages of domestication, wild-type dormancy mechanisms are still present in the seed.

According to Miller et al. (1964), all species of Limnanthes germinate in fall and have a requirement for cool weather during the growing season. In reports by Toy and Willingham, temperatures above 20°C inhibited

germination (1966) and caused secondary dormancy (1967). Cole (1974) has shown that light inhibits germination of Limnanthes alba at temperatures above the optimum for germination.

Seed dormancy in many seeds can be broken by a number of different methods, including prechilling (Stokes, 1954), temperature (Thompson, 1970; Harrington, 1923; Evenari, 1965), light (Isikawa, 1957; Vidaver, 1977), removal of seed coat (Rolston, 1978), increasing oxygen tension (Wareing and Foda, 1957), use of plant growth regulators (Ross, 1984) and leaching of chemical inhibitors (Wareing, 1965).

The objectives of this study were to: (1) determine the factors influencing germination of meadowfoam, (2) characterize the types of dormancy involved, and (3) develop methods of overcoming dormancy.

MATERIALS AND METHODS

Seed source

Meadowfoam seed lots used in this study included Limnanthes alba (Benth.) var. Mermaid, obtained from the 1984, 1985, and 1986 harvests; Limnanthes floccosa Howell from the 1983 harvest; and L. floccosa x L. alba grown in 1983.

Germination methods

Fifty seeds were placed on blotters saturated with deionized water in 10 x 10 x 2.5 cm clear plastic germination boxes with tightly fitting lids. The boxes were placed in germinators maintained within $\pm 1^{\circ}C$ of the desired temperature.

When necessary, light was provided by three 40-w cool-white fluorescent bulbs which provided a photoperiodic photon flux density (PPFD) of about 60 μmol $m^{-2}s^{-1}$. If required, darkness was provided by wrapping the boxes in aluminum foil.

Germination percentages were determined after 14 days with protrusion of the radicle from the seed coat as the criterion for germination. Seeds that failed to germinate were dissected and examined for viability.

Experiments were conducted in a randomized complete block design with four replications. Data were analyzed

by analysis of variance, and least significant differences were calculated to measure variation among treatment means.

Any deviations from these general procedures are described for the specific experiments.

Effect of temperature and light

Germination of 8 to 10-month-old seed of Mermaid-84, 20 to 22-month-old seed of L. floccosa, and 20 to 22-month-old seed of L. floccosa x L. alba was compared in light and dark at constant temperatures of 10, 15 and 20°C and alternating temperatures of 5-15, 10-20 and 15-25°C. The alternating temperature regime consisted of 16 h at the lower temperature and 8 h at the higher temperature. Light was provided during the high temperature portion of the cycle and for 8 h daily at the constant temperatures.

Effect of prechilling

Three-month-old seeds of Mermaid-85 were placed on moist blotters in germination boxes and held at 5°C for either 3 or 5 days in the dark. They were then transferred to 10 or 20°C to complete germination in the dark or with a daily 8-h light period.

Effect of plant growth regulators

Four concentrations each of gibberellic acid (GA), ethephon, kinetin, thiourea, and potassium nitrate

(KNO_3) were tested on Mermaid-84 at 15°C to determine the concentrations giving the highest germination percentage without producing abnormal seedlings. The most effective concentrations of three growth regulators were chosen for further study: 0.01 g L^{-1} kinetin, 0.5 g L^{-1} thiourea and 1 g L^{-1} KNO_3. Blotters were soaked in growth regulator solutions for 20 minutes before planting. Germination tests were conducted at 10, 15, 20, 5-15, 10-20 and 15-25°C in darkness. Tests were continued for 28 days to allow evaluation of normal and abnormal seedlings. Seed lots were 11 to 13-month-old seed of Mermaid-84 and 23 to 25-month-old seed of L. floccosa.

Effect of photoperiod

Two-month-old seeds of Mermaid-86 and 26-month-old seed of Mermaid-84 were germinated at 10 and 15°C in a growth chamber. Eight 40-w cool-white fluorescent and four 25-w incandescent bulbs provided a PPFD of 130-200 μmol $m^{-2}s^{-1}$ continuously. Each seed lot was exposed to a daily light period of 8, 16 and 24 h, with darkness imposed by wrapping the boxes in aluminum foil for the required dark periods.

Effect of the seed coat

Rate of water uptake by dormant and non-dormant seeds

Seed water uptake was measured in 25-month-old seed of Mermaid-84 and 1-month-old seed of Mermaid-86. One hundred seeds of each lot were placed on blotters saturated with water in germination boxes. Water uptake was measured after 0, 1, 3, 6, 12, 24, 36, 48 and 60 h at 10 and 20°C in light and dark. Following each imbibition period, seeds were removed, surface-dried with paper towels, and weighed immediately. The seeds were then dried at 103°C for 17 h and reweighed. Seed moisture content was calculated on the wet weight basis as follows:

$$\% \text{ Moisture} = \frac{\text{Wet Wt.} - \text{Dry Wt.}}{\text{Wet Wt.}} \times 100$$

Effect of mechanical scarification

Three-month-old seeds of Mermaid-85 were scarified by several methods. Seed coats were cut off with a razor blade at the radicle end, cotyledonary end, and both radicle and cotyledonary ends. Seeds of another group had the entire seed coat removed. Care was taken to avoid injury to the embryo. Seeds with coats intact served as a control. Twenty-five seeds from each treatment were germinated at 10, 15 and 20°C with an 8-h light period.

The experiment was designed as a completely randomized design with three replications.

Effect of increased oxygen tensions

The effects of higher oxygen tensions on germination were tested on intact seeds of 2-month-old Mermaid-86. Fifty seeds of each lot were placed on blotters saturated with deionized water or 1 g L^{-1} KNO_3 in 450 mL jars. The lid of each jar was fitted with a #7 two-hole rubber stopper with 7-mm glass tubes. The tubes were sealed with rubber septa. The jars were flushed for 3 minutes with O_2-N_2 mixtures containing 40, 60, 80 and 100% O_2. Flushing was repeated three times at an interval of three days. The gas mixture was introduced through hypodermic needles inserted through the septa. The proportions of O_2 and N_2 were regulated with a Matheson Flowmeter model 7351 H (Matheson Gas Products, Inc.) and monitored with a Percent Oxygen Monitor model 74223 (Bio-Tek Instruments, Inc., Burlington, VT). The seeds were placed at 10°C with daily 8-h light periods. Germination in air (21% O_2) was conducted in boxes. The experiment was repeated four times.

Effect of water-soluble seed extract

Water-soluble extract of whole seeds was obtained by leaching 10 g dry weight of 10-month-old seed of

Mermaid-85 in 100 mL of distilled water and soaking for 24 h at 15°C. The mixture was continuously stirred on a reciprocating shaker for 4 h and filtered using Whatman's No. 1 paper. The water-soluble extract was concentrated to 100, 200, 300 and 400 mL with a Buchler Flash Evaporator at 70°C. Twenty-two-month-old seeds of Mermaid-84 were germinated on blotters saturated with the water-soluble extract at 10°C with an 8-h light period. Seeds were considered germinated when both root and shoot were visible. Germination and shoot length were evaluated after 28 days.

RESULTS AND DISCUSSION

Effect of temperature and light

Germination of the three lots of meadowfoam was promoted by cool temperatures and inhibited by warm temperatures (Tables 1 and 2). The highest germination occurred at a constant temperature of 10°C and alternating temperatures of 5-15 and 10-20°C. Germination at 20°C caused a reduction in germination. When seeds were exposed to 15 and 20°C for only 8 h in alternating temperature regimes, however, any inhibitory effects by these warm temperatures were overcome by returning the seeds to 5 and 10°C for 16 h each day. Further work will be needed to ascertain whether temperature alternations _per se_, as opposed to average temperatures or total degree days, promoted germination. These findings agree with Toy and Willingham (1966) and Cole (1974), who demonstrated that germination of several species of _Limnanthes_ was inhibited by temperatures above an optimum of 10-12°C.

Daily 8-h light exposures also had an inhibitory effect on germination (Tables 3 and 4). The inhibition was minimal at the favorable cool temperatures of 10, 5-15 and 10-20°C but was more severe at warmer temperatures. Cole (1974) also reported on the inhibitory effects of light on meadowfoam germination. In this respect

meadowfoam is similar to other light-inhibited seeds such as Nemophila insignis (Black and Wareing, 1959).

Limnanthes floccosa was more dormant than either L. alba or the cross of L. floccosa x L. alba. This would indicate that seed dormancy is under genetic control and could be reduced during the development of future cultivars.

Effect of prechilling

Nearly complete germination was obtained at 10°C after 5 days prechilling at 5°C (Table 3). Prechilling also promoted germination at the otherwise inhibitory temperature of 20°C. The seeds used in this test were recently harvested and did not germinate at 20°C without prechilling. A 3-day exposure to 5°C promoted germination considerably while a 5-day period was still more beneficial. Prechilling did not remove the inhibitory effect of light. Additional tests are needed to determine the effects of longer prechilling periods and other temperatures.

Effect of plant growth regulators

Effects of three growth regulators on germination of Mermaid and L. floccosa are shown in Tables 4 and 5. Preliminary tests had determined that concentrations of 0.05 g L^{-1} kinetin, 0.5 g L^{-1} thiourea and 1.0 g L^{-1}

potassium nitrate (KNO_3) had possible stimulatory effects without causing abnormal seedlings. No stimulatory concentrations of gibberellic acid or ethephon were found.

Thiourea and KNO_3 increased the germination of L. floccosa at each constant and alternating temperature, while kinetin had no effect. The Mermaid seed, being less dormant, germinated well in water at the lower temperatures and was not benefitted by growth regulators. Germination was increased at 20 and 15-25°C, but not to a practical degree. Kinetin again was ineffective on Mermaid.

The benefits of growth regulators in promoting germination would be expected to be greater on seed lots with deeper dormancy levels.

Potassium nitrate appears to be the most effective chemical germination promoter. Potassium nitrate is in common use in seed testing laboratories and is especially beneficial on light-promoted seeds such as grasses. In the case of meadowfoam, it also promotes germination of light-inhibited seeds.

Effect of photoperiod

Photoperiod had a marked influence on germination of both old and new seed of Mermaid (Fig. 1). The highest germination occurred under continuous darkness, while each

increase in the daily light period caused a corresponding decrease in germination. In this respect meadowfoam is similar to other dark-favored seeds such as Nigella and Eschscholzia (Isikawa, 1953), and Nemophila insignis (Black and Wareing, 1959) in which the absolute length of the light plays a determinative role. The 2-month-old seed of Mermaid-86 was still dormant and germination was completely inhibited by even an 8-h daily light period.

Photoperiodic responses were similar at 10 and 15°C for 25-month-old seed of Mermaid-84 (Fig. 2).

Seed coat effect

Meadowfoam has a thick, tough seed coat and it was postulated that dormancy could be due to restriction of water or oxygen by the seed coat or presence of germination inhibitors. A series of experiments was conducted to explore these possibilities.

Mechanical scarification

The effects of several scarification procedures on germination of 3-month-old seed of Mermaid-85 are summarized in Table 6. Removing a piece of seed coat at both the cotyledonary and radicle ends led to nearly complete germination, while removing the seed coat from only one location was not as effective. Removal of the entire seed coat increased germination over that of intact

seeds, but not to the extent that chipping the seed coat did. The reason for this is not clear but it may have been caused by mechanical injury or the presence of microorganisms on the unprotected embryo.

Removal of the seed coat promoted additional germination at 10 and 15°C, but did not overcome the inhibition by temperature at 20°C (Fig. 3). This would indicate that the causes of dormancy in meadowfoam may lie both in the embryo and the seed coat.

Further experiments were conducted to determine whether the beneficial effects of seed coat removal could be attributed to improved water absorption or gas exchange.

Water uptake

No differences were detected in rate of water uptake by dormant and non-dormant seeds (Fig. 4), in favorable and unfavorable temperatures (Fig. 5) or in light or dark (Fig. 6). These data suggest that the seed coat is not a barrier to water uptake by dormant seeds. Factors other than water-impermeability of seed coat must be responsible for the seed dormancy in meadowfoam.

Oxygen permeability

An atmosphere of 100% O_2 promoted an increase in germination of 2-month-old dormant seed from 48 to 64%

(Table 7). It is thus possible that the seed coat forms at least a partial barrier to diffusion of oxygen or other gases.

Germination in a substrate of 1 g L^{-1} KNO_3 also raised the germination to 64% (Table 8). The effects of KNO_3 and increased O_2 were additive, with a germination of 90% obtained from KNO_3 and 100% O_2.

It would be fully consistent with the experimental results to suggest that perhaps a germination inhibitor is present in the seed coat and that cutting the seed coat in various positions and in 100% O_2 brings about the destruction of the inhibitor.

Further experiments were conducted to evaluate the inhibitory effects of water-soluble extract on non-dormant seeds.

Effect of water-soluble seed extract

To test the possibility that the seed contains germination inhibitors, 22-month-old seeds of Mermaid-84 were germinated on moist blotters saturated with water-soluble extract from 10-month-old seed of Mermaid-85. Germination was significantly reduced by the water-soluble extract, suggesting the presence of an inhibitor (Fig. 7). Root and shoot development were retarded and decreased progressively with increase in extract concentration. These results suggest that

water-soluble inhibitors are present in the seed coat or embryo, even in non-dormant seeds, and could play a role in the dormancy mechanism.

Since the extract from 10-month-old seeds caused inhibition of germination of other seeds, further work should be done on changes in inhibitor concentration as dormancy increases with age.

Elliot and Leopold (1953) showed that the inhibitory influence of the oat covering structures can be attributed to the presence of a water-soluble germination inhibitor in the husk. Meadowfoam shows a similar type of dormancy, indicating the inhibitors in the seed coat may be important in controlling dormancy.

CONCLUSIONS

A number of the treatments imposed on meadowfoam seed overcame dormancy partially or fully, suggesting that there are several mechanisms regulating dormancy. Evidence from this study suggests that the seed coat is partially responsible, acting as a barrier to diffusion of oxygen and because it is a source of germination inhibitors. Cutting and removing portions of the seed coat was an effective treatment for removing dormancy, suggesting that mechanical scarification could be a practical possibility for breaking dormancy of bulk lots of seed. Embryo dormancy is also implicated in that treatment with KNO_3 was very effective in overcoming dormancy, alone or in combination with oxygen.

For routine germination testing of meadowfoam, it is recommended that seeds be planted on a substrate moistened with 1 g L^{-1} KNO_3 and placed in dark at 10°C for 14 days. However, these procedures will not promote germination of all seeds of freshly harvested seed lots. If circumstances warrant, complete germination of dormant seed lots can be obtained by germination on a substrate of KNO_3 in an atmosphere of 100% O_2.

Meadowfoam is an excellent species on which to conduct further studies on the physiological effects of light intensity, light duration, light quality, oxygen concentration and germination inhibitors.

Table 1. Effect of constant temperatures on germination percentage of three seed lots of meadowfoam in light and dark.

Seed lot	Germination temperature (°C)					
	10		15		20	
	Light	Dark	Light	Dark	Light	Dark
	% germination					
L. floccosa x *L. alba*	79c*	88b	47bc	65a	9d	24ab
L. alba var. Mermaid-84	86b	91a	44bc	70a	12cd	27a
L. floccosa	59e	68d	37c	52b	17bc	24a

* Means followed by the same letter within columns for each germination condition are not significantly different at L.S.D. 0.05.

Table 2. Effect of alternating temperatures on germination percentage of three seed lots of meadowfoam in light and dark.

Seed lot	Germination temperature (°C)					
	5-15		10-20		15-25	
	Light	Dark	Light	Dark	Light	Dark
	% germination					
L. floccosa x *L. alba*	89a*	90a	80a	90b	8a	33c
L. alba var. Mermaid-84	91a	94b	91b	91b	8a	25b
L. floccosa	76c	62d	74c	69c	5a	19b

* Means followed by the same letter within columns for each germination condition are not significantly different at L.S.D. 0.05.

Table 3. Effect of prechilling at 5°C on germination percentage of 3-month-old seed of Mermaid-85 in light and dark.

	Germination temperature (°C)			
	10		20	
Prechill duration	Light	Dark	Light	Dark
days	% germination			
0	62d*	67c	0g	0g
3	70c	78b	20f	40e
5	81b	90a	38e	60d

* Means followed by the same letter are not significantly different at L.S.D. 0.05.

Table 4. Effect of plant growth regulators on germination percentage of 11-month-old seed of Mermaid-84 and 23-month-old seed of L. floccosa at constant temperatures.

Growth regulator	Concentration	Mermaid-84			L. floccosa		
		Temperature °C					
		10	15	20	10	15	20
	g L-1	% germination					
Water		85b*	55ab	12c	49c	28c	7c
Kinetin	0.01	81b	46b	11c	52c	32c	11c
Thiourea	0.5	91a	59ab	20b	68b	50b	26b
Potassium nitrate	1.0	95a	67a	35a	84a	68a	38a

* Means followed by the same letter within columns for each germination condition are significantly different at L.S.D. 0.05.

Table 5. Effect of plant growth regulators on germination percentage of 13-month-old seed of Mermaid-84 and 25-month-old seed of L. floccosa at alternating temperatures.

Growth regulator	Concentration	Mermaid-84			L. floccosa		
		Temperature °C					
		5-15	10-20	15-25	5-15	10-20	15-25
	g L^{-1}	% germination					
Water		91a*	91a	18ab	56b	61b	11b
Kinetin	0.01	88a	93a	11b	52b	56b	1c
Thiourea	0.5	96a	95a	28a	82a	83a	21a
Potassium nitrate	1.0	95a	97a	25a	85a	87a	26a

* Means followed by the same letter within columns for each germination condition are significantly different at L.S.D. 0.05.

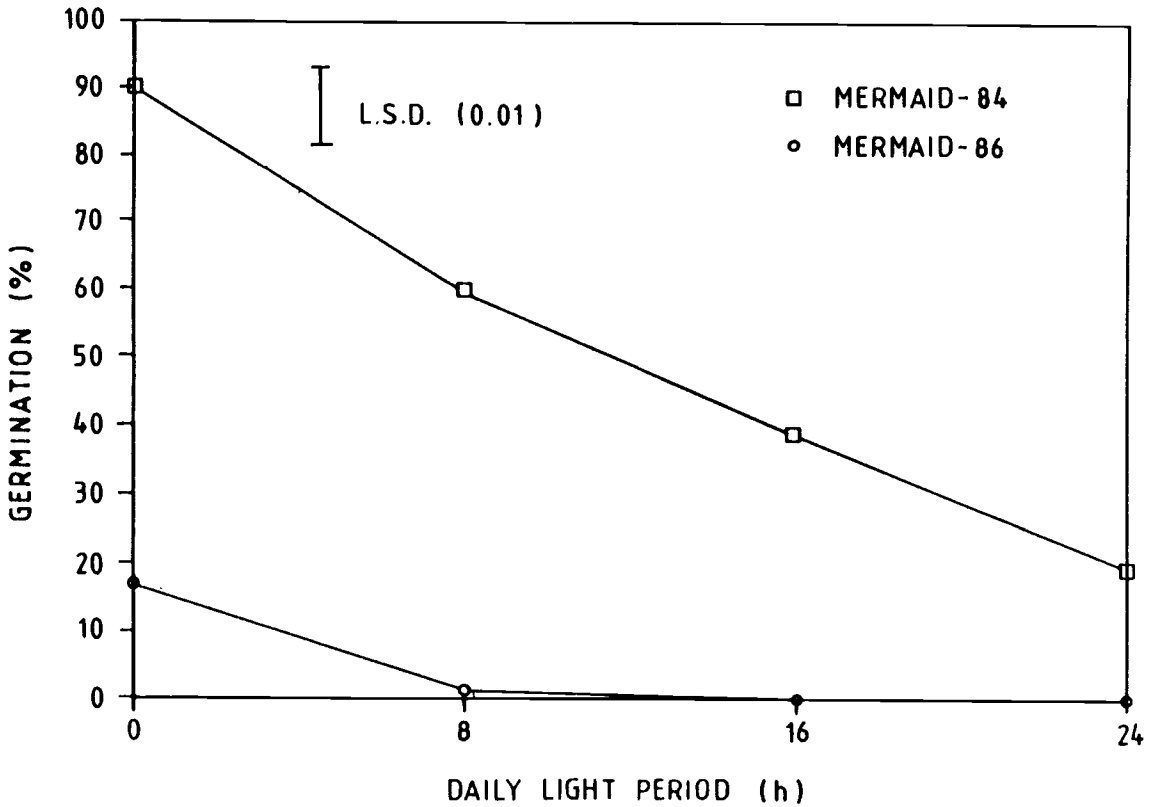

Fig. 1. Effect of day length on germination of 25-month-old seed of Mermaid-84 and 2-month-old seed of Mermaid-86 at 10°C in darkness.

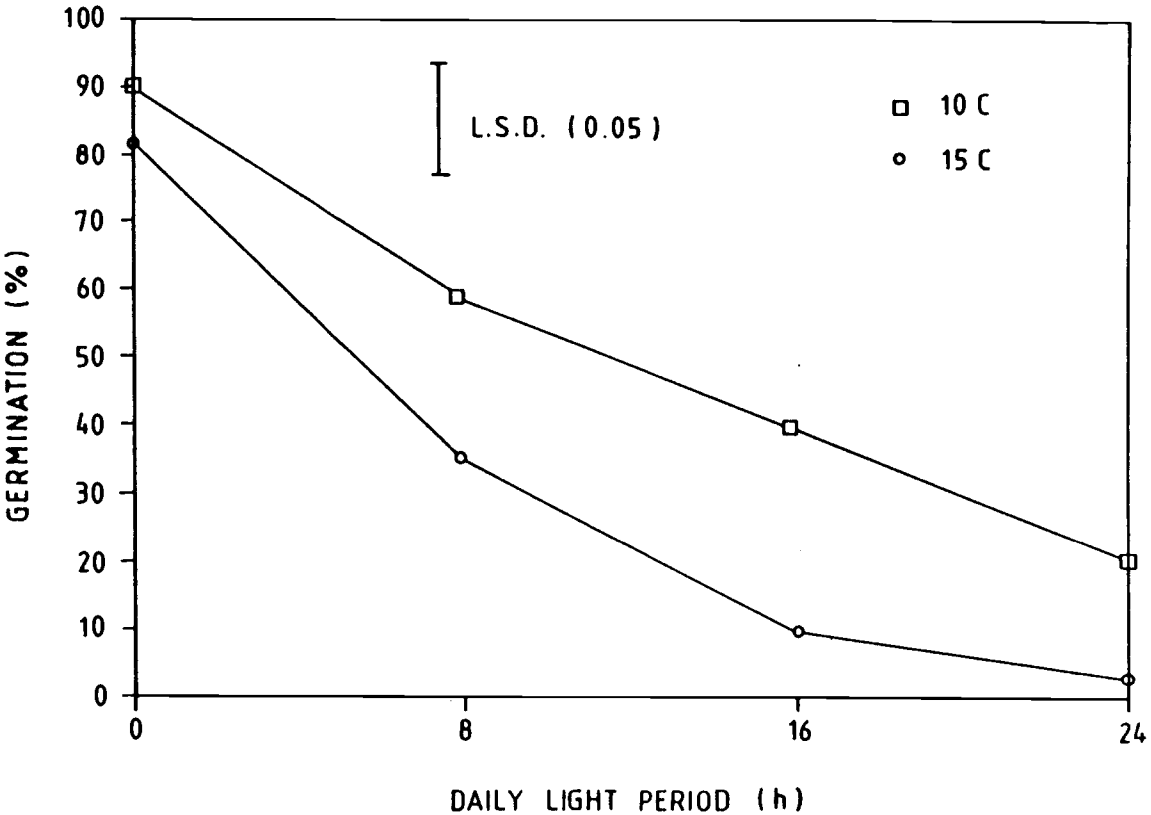

Fig. 2. Effect of day length on germination of 25-month-old seed of Mermaid-84 at 10 and 15°C.

Table 6. Effect of cutting and removal of the seed coat on germination of 3-month-old seed of Mermaid-86 at 10°C.

Seed coat treatment	Germination
	%
Intact seed	48a*
Seed coat removed	75bc
Cotyledonary end cut	73b
Radicle end cut	81c
Cotyledonary and radicle end cut	92d

* Means followed by the same letter are not significantly different at L.S.D. 0.01.

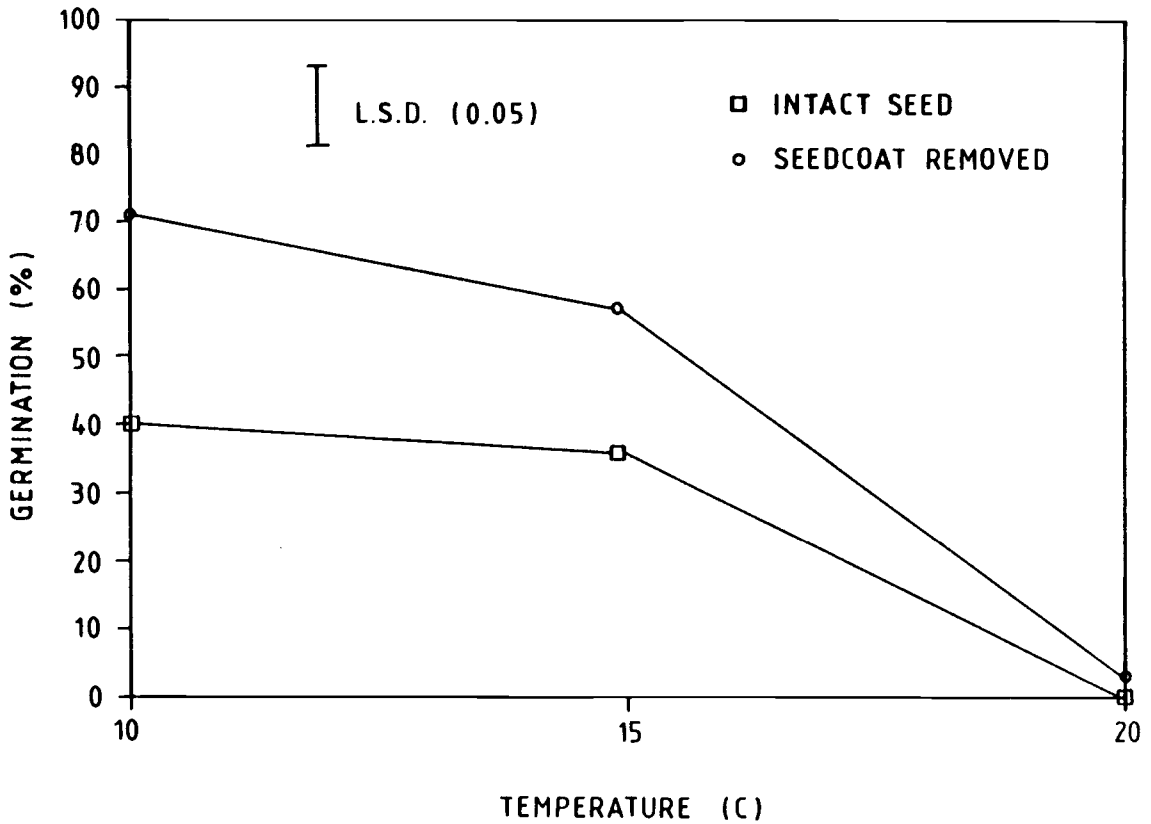

Fig. 3. Effect of seedocat removal on germination of 3-month-old seed of Mermaid-85 at three temperatures in darkness.

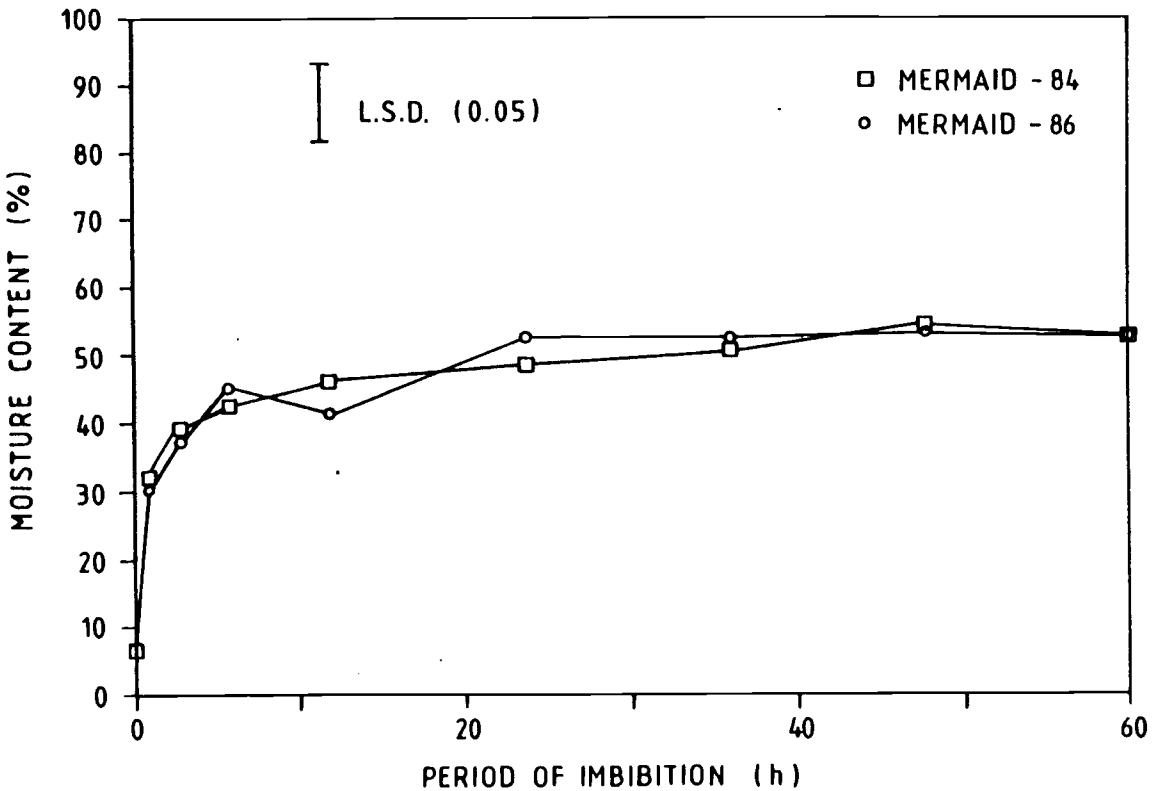

Fig. 4. Time course of water uptake by non-dormant (1984 harvest) and dormant (1986 harvest) Mermaid seed at 10°C.

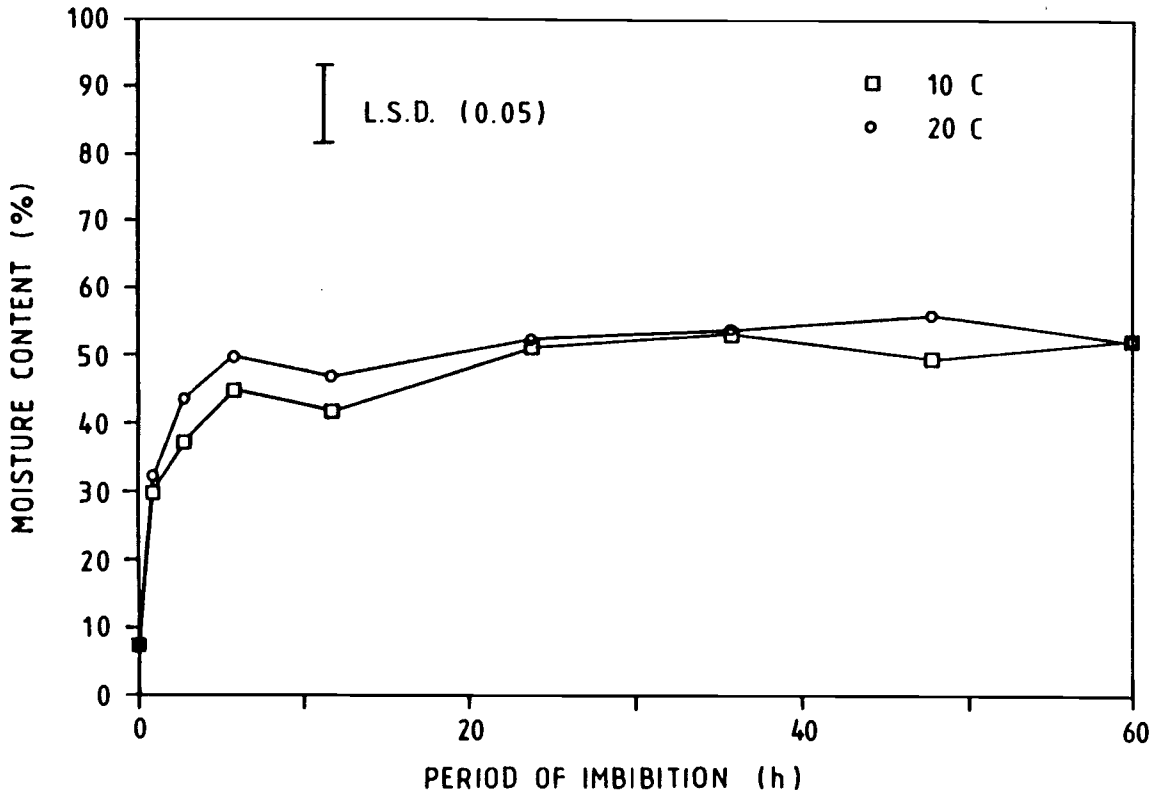

Fig. 5. Effect of temperature on water uptake of dormant seeds of L. alba var. Mermaid (1986 harvest).

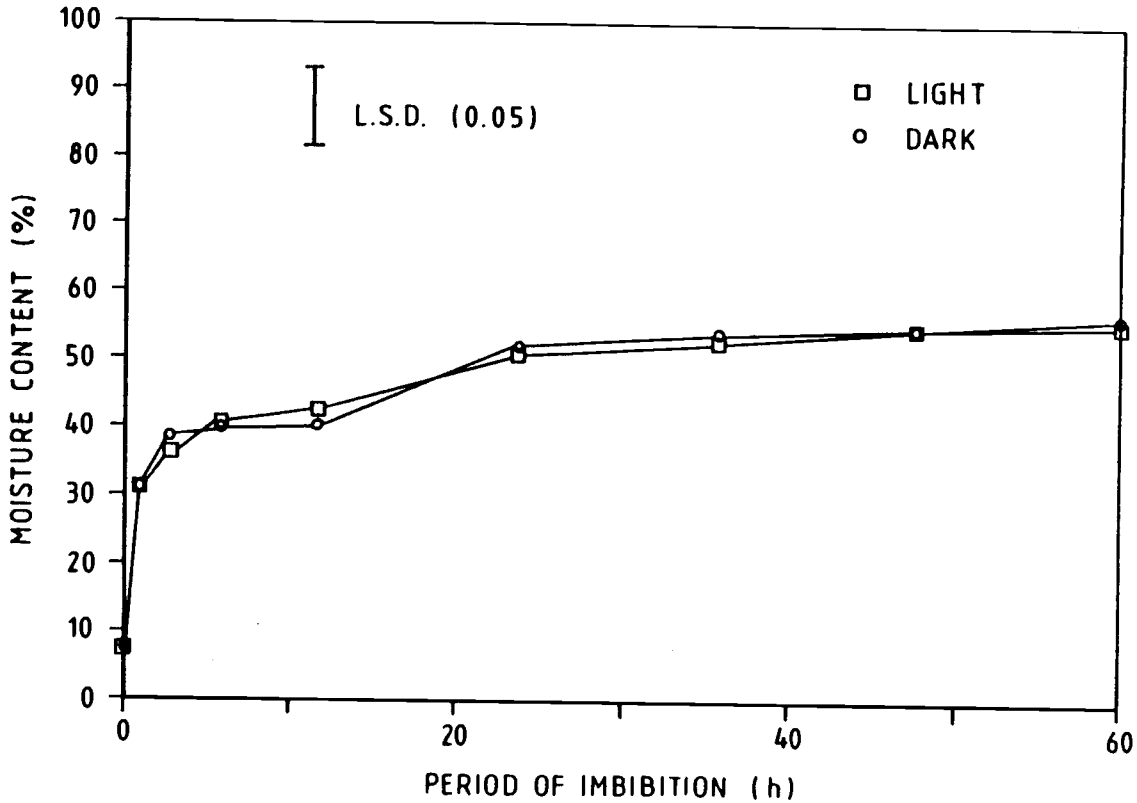

Fig. 6. Rate of water uptake in dormant seed of Mermaid-86 (30 days after harvest) at 10°C in light and dark.

Table 7. Effect of 1 g L^{-1} KNO_3 and oxygen on germination of 2-month-old seed of Mermaid-86 at 10°C in darkness.

Oxygen Concentration	Moistening agent	Germination
%		%
21 (air)	water	15a*
100	water	48b
21 (air)	KNO_3	64c
60	KNO_3	82d
80	KNO_3	89d
100	KNO_3	90d

* Means followed by the same letter are not significantly different at L.S.D. 0.05.

Table 8. Effect of oxygen concentration on germination percentage of scarified and non-scarified seeds of 2-month-old Mermaid-86.

Oxygen concentration	Non-scarified	Scarified
%	% germination	
21 (air)	12e	44c
40	35d	46bc
60	43bcd	48bc
80	48bc	57ab
100	49bc	64a

* Means followed by the same letter are not significantly different at L.S.D. 0.05.

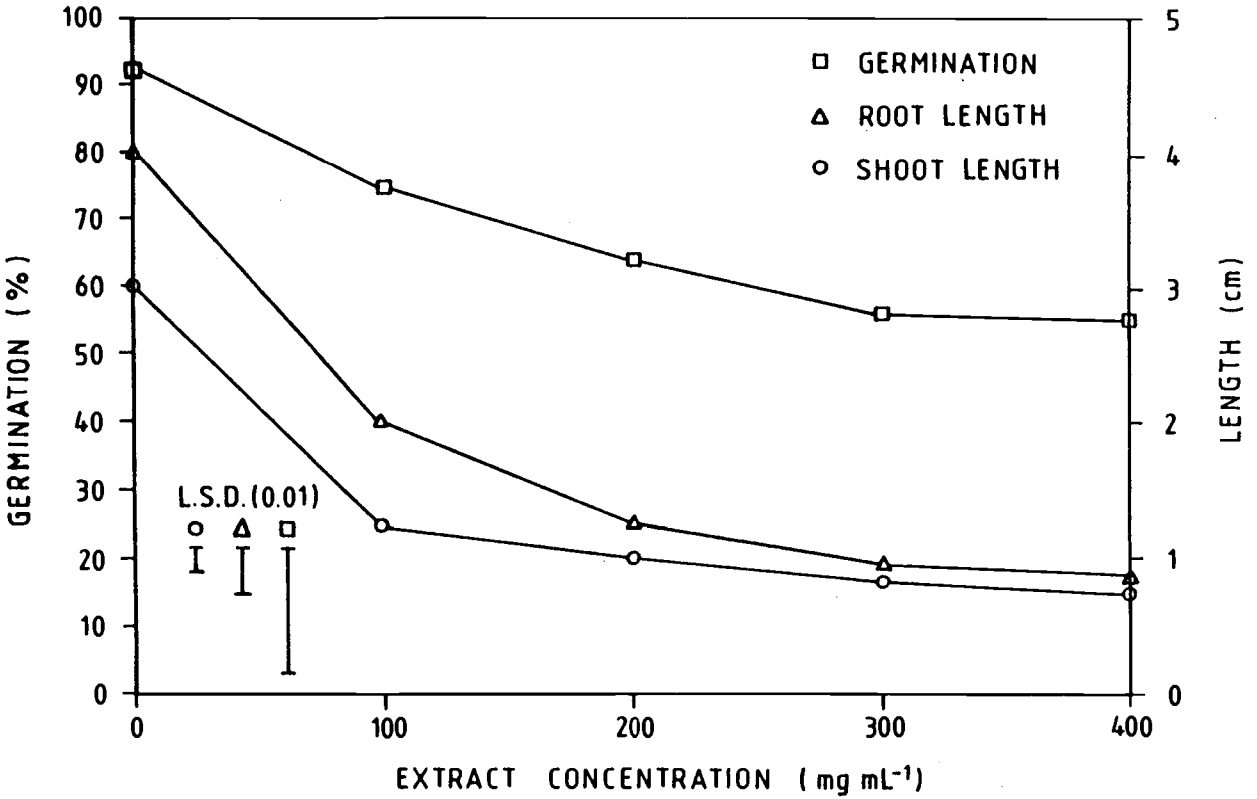

Fig. 7. Effect of water-soluble extract from 10-month-old seed of Mermaid-85 on germination and seedling growth of 22-month-old seed of Mermaid-84 at 10°C.

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APPENDIX

Analysis of Germination Data

Two experimental designs were used: completely randomized and randomized complete block.

Completely randomized design

Limited germinator space necessitated the use of a completely randomized design. Four replications were arranged factorially. No effort was made to confine treatments to any particular position of the germinator.

Randomized complete block design

Since a temperature and light gradient was assumed, we used a randomized complete block design. The treatments were randomly assigned within each replication.

Analysis

Data was analyzed statistically by analysis of variance. When the F-test was significant, the Least Significant Difference (LSD) was calculated at the 5% level. LSD at the 5% level was calculated using the formula:

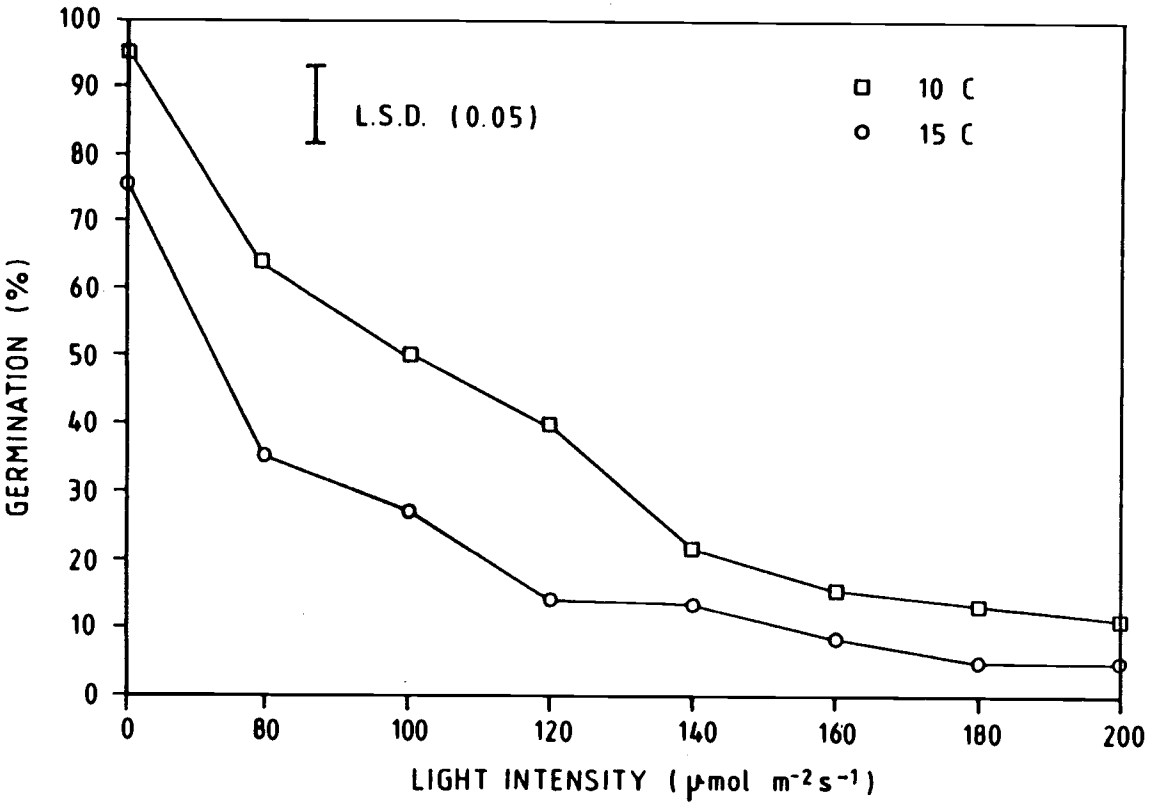
$$LSD_{0.05} = t_{0.05} \times \left(\frac{2\ EMS}{n} \right)^{\frac{1}{2}}$$

where t is the student-t value, EMS the error mean square, and n the number of observations.

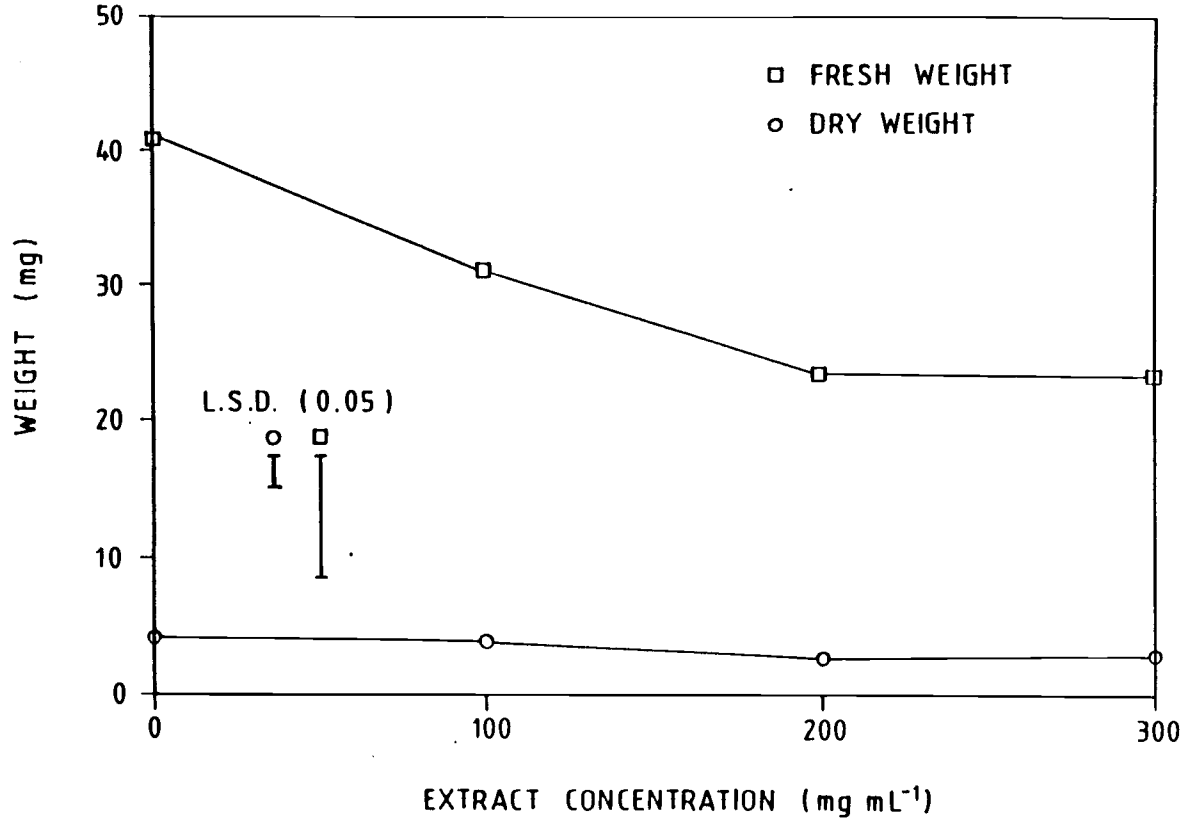
For completeness all means were presented. LSD comparisons were made only with those means associated with significant F-tests.

Appendix Table 1. Effects of concentration of plant growth regulators on germination of 22-month-old seed of Mermaid-84 at 15°C.

Growth regulator	Concentration	Germination	
		Normal	Abnormal
	g L^{-1}	%	
Water		46	0
Gibberellic acid	0.05	26	4
	0.1	10	6
	0.2	4	22
	0.4	0	22
Ethephon	0.12	20	10
	0.23	8	16
	0.46	0	22
	0.93	0	24
Kinetin	0.01	42	2
	0.02	26	14
	0.03	8	16
	0.04	8	26
Thiourea	0.5	54	2
	1.0	50	4
	2.0	42	12
	3.0	40	18
Potassium nitrate	1.0	60	0
	2.0	50	2
	3.0	30	6
	4.0	0	14



Appendix Fig. 1. Effect of light intensity on germination of 25-month-old seed of Mermaid-84.



Appendix Fig. 2. Effect of water-soluble extract from 10-month-old seed of Mermaid-85 on dry weight and fresh weight of 22-month-old seed of Mermaid-84.