PHYSIOLOGICAL STUDIES ON DORMANCY IN CAPE TULIP

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Summary

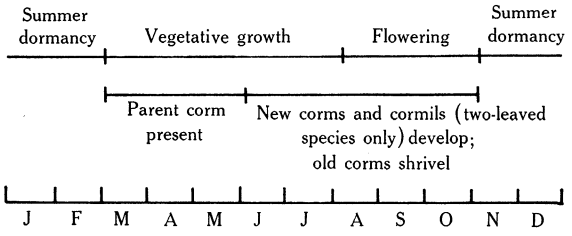
A high proportion of cormils of the two-leaved species of the weed, Cape tulip, showed long-term dormancy, and experiments showed that this dormancy is not due to a high level of endogenous growth inhibitors but is due to physical factors associated with the presence of intact cormil coats. Dormancy is broken if the thin and papery inner cormil coat is ruptured, and observations suggest that this coat is impermeable to water and probably oxygen and carbon dioxide as well. The outer cormil coat is not impermeable to these substances but is thick and heavily lignified and thus protects the fragile inner coat.

It is suggested that field treatments such as burning-off and cultivation in autumn, which break long-term dormancy of Cape tulip, probably act by rupturing or destroying cormil coats.

I. INTRODUCTION

Cape tulip is the common name given to the poisonous weeds *Homeria breyniana* (L.) Lewis (one-leaved species) and *H. miniata* Sweet (two-leaved species). These species are native to South Africa but were introduced into Australia and New Zealand as garden plants. They rapidly spread from introduction sites and are now regarded as serious pests because all plant parts, whether fresh or dried, are poisonous to stock.

The life cycle of the weed is summarized in the accompanying diagram. Both species are propagated vegetatively from corms which usually emerge from summer dormancy in March or April, after opening rains.



Eradication of these weeds has been hindered by prolonged corm dormancy (failure to sprout under apparently favourable conditions), thus necessitating long-term eradication programmes. Walker (1954), for example, reported that usually more than 60% of the corms of *H. breyniana* show long-term dormancy. In practice, this long-term dormancy is overcome by burning-off surface cover in summer or autumn, or by cultivating in autumn (Orchard 1957; Parsons 1958; Pearce 1968).

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As the growing season advances, the non-dormant corm is depleted of food reserves and shrivels, whilst immediately above it a new corm is formed by enlargement of an internode. In the two-leaved species, lateral corms and cormils also rise in axils of scale leaves and at each node of the flowering stalk, whilst only lateral corms are formed in the one-leaved species.

The aim of the present investigation was to determine whether long-term dormancy of cormils of the two-leaved species of Cape tulip (*H. miniata*) is due to chemical or physical factors.

II. Methods and Results

(*a*) *Source of Cormils and Observations on Dormancy*

Cormils were collected from a single location at Yatala Vale, S.A., on March 11, May 11, and July 16, 1960; typical sprouted and non-sprouted cormils are shown in Figure 1. In the laboratory, the cormils were washed, surface-sterilized in 0·01% $HgCl_2$ solution for 3 min, rinsed well in distilled water, and blotted dry. Dried cormils were stored in a screw-lid glass jar at room temperature.

The percentage of cormils which showed long-term dormancy was measured in a pot experiment. Cormils were sown at a depth of 5 mm in soil at the rate of 40 cormils per pot (4 in. diam.) and mean plant emergence was measured 2·5 months later. The following tabulation shows that a large percentage of the cormils remained dormant in this test, even though they appeared viable on dissection:

Date Collected	Date Sown	Description of Outer Coat	Percentage Cormils Sprouted per Pot
11.iii.60	22.iv.60	Highly lignified	20
11.v.60	12.v.60	Highly lignified	0
		Poorly lignified	0
16.vii.60	18.vii.60	Highly lignified	0

Non-sprouted intact and dissected cormils, as well as sprouted cormils in various stages of development, are shown in Figure 2. Except where stated, these experiments were carried out only on cormils possessing heavily lignified outer coats, and pronounced development of the bud was taken to indicate sprouting.

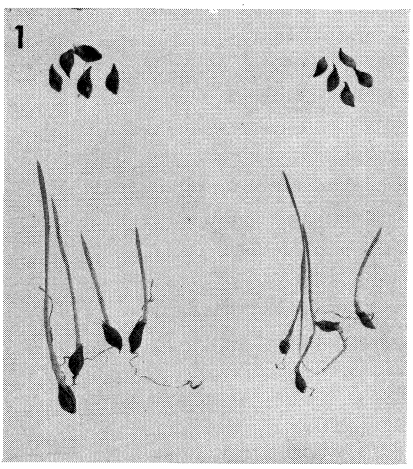
Field observations on cormil dormancy were also made at Yatala Vale on July 16, 1960, when cormils would normally have sprouted. At shallow depths, e.g. 1 in., about 95% of cormils had sprouted, but in the 1–5-in. zone greater than 50% of the cormils were dormant.

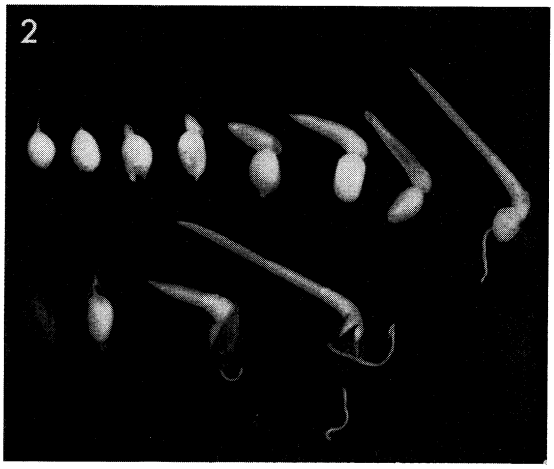
(*b*) *Anatomical and Microchemical Studies*

Unsprouted cormils were fixed in formalin–glacial acetic acid–50% ethanol (5 : 5 : 90 v/v), dehydrated by the t-butanol method (Johansen 1940), and infiltrated and embedded in paraffin wax. Prior to mounting, microtome sections were stained with safranin for 48 hr and counterstained with fast green for 5 sec. Microchemical tests for starch, lignin, fats, cutin, and suberin were also carried out on fresh tissues as described by Johansen (1940).

Each cormil was found to be surrounded by two coats (Fig. 3). The outer coat was usually highly lignified and made up of long, narrow cells which were extremely

tightly packed. The degree of lignification of this coat was indicated by safranin staining. Inner layers of thin-walled, non-lignified cells stained with fast green. Differential staining showed that dark-brown cormils were surrounded by a thicker, more highly lignified coat than were light-brown cormils. However, the degree of lignification of the outer coat was not uniform and the scar tissue at the base of each cormil, the point at which it was attached to the parent plant, tended to be poorly lignified. Further, the outer lignified coat was not continuous as it was usually segmented into three contiguous parts at the apex of the cormil immediately above the bud (see Fig. 2). The developing bud forces its way through the outer coat at this segmented area.





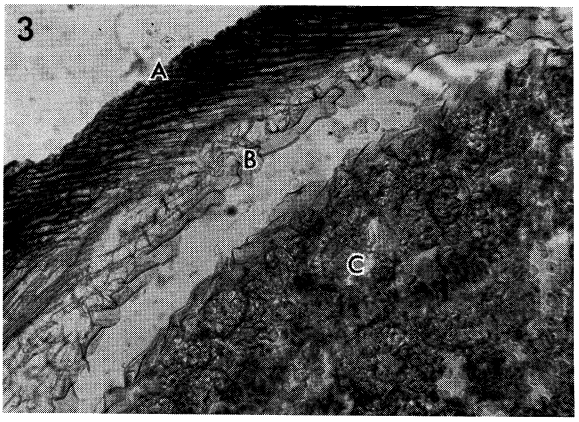


Fig. 1.—Heavily and poorly lignified cormils. $\times 0{\cdot}76$.

Fig. 2.—Sprouting of intact and dissected cormils. $\times 2{\cdot}6$.

Fig. 3.—Longitudinal section of a mature, non-sprouted cormil. *A*, outer coat; *B*, inner coat; *C*, inner cormil tissue. $\times 90$.

The inner coat of each cormil was white and paper-like and consisted of a continuous layer of large, convoluted cells (Fig. 3). This coat was usually attached to, or in close proximity to, the outer lignified coat, but it was possible, with care, to dissect away the outer coat without damaging the inner coat. This was most easily done when the outer coat was soft and poorly lignified. Microchemical tests showed that the inner coat contained lignin, cutin, and fat globules.

The inner cormil tissue consisted of large, thin-walled parenchyma cells filled with starch grains. Fast green was taken up by these cells and their contents.

The chemical composition of non-sprouted corms of both species was similar to that of cormil tissues in all respects. Inner corm tissue contained abundant starch; the inner coat, which was thin and papery, contained lignin, cutin, and fat, and the outer coat was fibrous. However, outer coats of corms were reticulate whereas those of cormils were almost continuous.

(*c*) *Dissection Treatments*

The aim of these experiments was to compare the sprouting of intact and partially and completely dissected cormils (both coats removed) under moist conditions in darkness at 25°C.

It is evident from Table 1 that sprouting of cormils was markedly increased when outer and inner cormil coats were removed. Removal of a piece of the outer coat did not stimulate sprouting but complete removal of this coat did to a minor extent. Since the presence of an intact inner coat markedly inhibited sprouting, it is probable, in those cases where removal of the outer coat stimulated sprouting, that the inner coat had also been ruptured.

TABLE 1

INFLUENCE OF DISSECTION TREATMENTS ON SPROUTING OF CORMILS

Values are expressed as percentage cormils sprouted after 17 days

Date Cormils Collected	Cormils Sprouted after Dissection Treatment Indicated:			
	Intact	Minus Piece of Outer Coat	Minus Outer Coat	Minus Outer and Inner Coats
11.iii.60	15·0	—	—	78·3
11.v.60	0	—	—	60·0
16.vii.60	0	0	12·5 40·0*	35·0

* Inner coat removed after 17 days and subsequent sprouting recorded after a further 21 days.

Although dissection resulted in increased sprouting of cormils, the percentage of cormils sprouting decreased with successive collections. It is suggested that this occurred because cormils incapable of sprouting constituted a progressively increasing proportion of each cormil collection. Apart from a low percentage in the first collection, no intact cormils sprouted.

(*d*) *Chemical versus Physical Factors in Cormil Dormancy*

Although removal of cormil coats stimulated sprouting, further experimentation was necessary to determine the relative importance of chemical and physical factors in cormil dormancy.

(i) *Levels of Endogenous Growth Regulators in Cormils*

Measurements of levels of endogenous growth promoters and growth inhibitors in mature, non-sprouted cormils collected on March 11, 1960 and immature, developing cormils (attached to parent plants) harvested on July 11, 1960 were made using a wheat coleoptile straight-growth test. Immature cormils were extracted with diethyl ether only, but mature cormil tissue was extracted with both ether and distilled water. Methods of extraction and fractionation were according to Hemberg (1958) or as given in Table 2. Following extraction and fractionation, fractions were chromatographed in duplicate on paper (Whatman No. 1) at 23°C in isopropanol–ammonia–water (10 : 1 : 1 v/v) using a one-way ascending technique. Dried chromatograms were divided into 15 pieces 1 by 3 cm in area, each of which was cut into small pieces and placed in a vial containing 1·5 ml 0·02M NaH_2PO_4 solution, pH 5·0. Standard solutions of indoleacetic acid (10^{-4} and 10^{-5}M) and *trans*-cinnamic acid ($10^{-3\cdot5}$M) were included as well as controls; blank pieces of Whatman No. 1 paper were used in the latter. After elution of the pieces for 1 hr at 25°C in darkness, 10 internode sections of wheat coleoptiles (*cv.* Warigo or Javelin) were added to each vial, and vials were sealed and placed on a reciprocating shaker for 18–19 hr in darkness at 25°C. Section lengths were then measured with a micrometer scale fitted to a binocular microscope. Section length was expressed as a percentage of the mean of 30 control sections.

The second method used was that of cormil bioassay. Extracts were chromatographed as described above; chromatograms were divided into 15 pieces, and each piece was added to a small Petri dish (4·0 cm diam.) containing 0·5 ml 0·02M NaH_2PO_4 solution. In the case of hexane and hot water fractions (Table 2), each aliquot was uniformly applied to a 1 by 3-cm piece of chromatography paper and dried before assay. Five cormils, minus their coats, were placed in a horizontal position on each paper strip. Only cormils which had well-developed terminal buds were used and control strips and a cinnamic acid standard were included as well. Petri dishes were placed in a moist atmosphere at 25°C for about 30 days, after which counts of sprouted cormils were made.

(1) *Results of Wheat Coleoptile Straight-growth Test.*—Bioassay results of acidic ether extracts of mature and immature cormils (Fig. 4) show that little promoter or inhibitor activity was present in non-sprouted cormils. Similarly, little growth regulator activity was detected in acetonitrile, hexane, and water fractions of an extract of mature, non-sprouted cormils (e.g. Fig. 4). In contrast, immature cormils contained a highly active growth-promoting zone (same R_F as indoleacetic acid) and a highly active β-inhibitor zone (R_F 0·35–0·65).

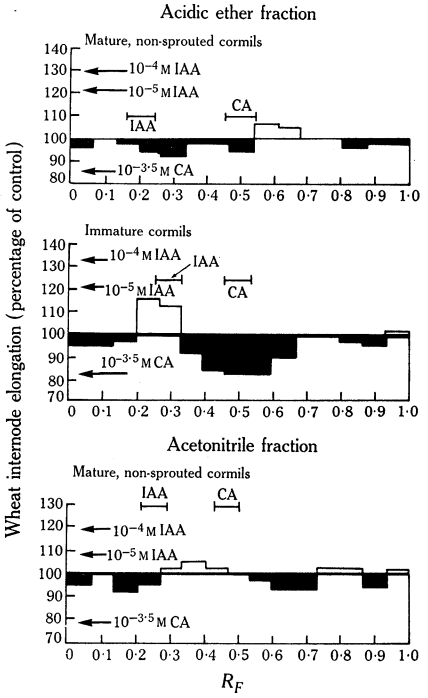


Fig. 4.—Levels of endogenous growth regulators in cormils. *IAA*, indoleacetic acid; *CA*, *trans*-cinnamic acid.

(2) *Results of Cormil Bioassay.*—When acetonitrile, hexane, and water fractions of the aqueous extract of mature, non-sprouted cormils were tested in the cormil

TABLE 2

EXTRACTION AND FRACTIONATION OF CORMIL TISSUE

16·8 g finely ground, freeze-dried material extracted with 30 ml triple-distilled water for 48 hr at 4–5°C (three changes of water made)

Aqueous extract filtered through Whatman No. 1 paper and evaporated to dryness *in vacuo* at 30°C; brown residue left

Acetonitrile (30 ml) and hexane (30 ml) added, shaken well, and layers allowed to separate

Acetonitrile fraction divided into two and chromatographed		Hexane fraction divided into two, evaporated to dryness, and taken up in 1·5 ml 0·02M NaH_2PO_4 solution		Brown, sticky residue taken up in 20 ml hot water	
Wheat coleoptile bioassay	Cormil bioassay	Wheat coleoptile bioassay	Cormil bioassay	2-ml aliquot and 1 : 10 and 1 : 100 dilutions tested in wheat coleoptile bioassay	2-ml aliquot tested in cormil bioassay

bioassay all cormils sprouted, indicating that no growth-inhibiting substances were present.

These bioassay results strongly suggest that cormil dormancy is not due to the presence of high levels of endogenous growth inhibitors.

(ii) *Use of Dormancy-breaking Chemicals*

Cormils were treated with ethylene chlorohydrin (0·1%) for varying lengths of time, and various concentrations of gibberellic acid and glutathione. They were then incubated in the dark for at least 30 days at 25°C. None of the three compounds tested were effective in overcoming cormil dormancy, and in most treatments none of the cormils sprouted.

(iii) *Leaching Treatments*

(1) *Water Treatment.*—In order to determine whether sprouting could be increased following the removal of possible water-soluble growth inhibitors, cormils were leached for 1, 2, or 3 days, in 4 ml, 500 ml, or a stream of running water. Leached cormils were placed on moist filter paper in Petri dishes in darkness at 25°C and counts of sprouted cormils were made 4 months later. None of the leached cormils sprouted.

(2) *Acid Treatment.*—Batches of 100 cormils were immersed in conc. H_2SO_4 for $1\frac{1}{2}$, $3\frac{1}{2}$, or 6 min. After treatment, cormils were thoroughly rinsed in tap water, blotted dry, and stored air-dry in stoppered vials at room temperature. After 80 days, acid-treated and control cormils were dissected and counts of cormils with enlarged buds were made. Data in the following tabulation show that sulphuric acid treatment resulted in a significant increase ($P < 0{\cdot}02$ in χ^2 test) in the number of cormils with enlarged buds. Furthermore, these cormils were still viable as they sprouted when placed in a moist environment.

Length of acid treatment (min)	0	1·5	3·5	6
Percentage of cormils with enlarged buds	27	46	34	45

Although this treatment could have removed acid-soluble growth inhibitors from cormil coats, it is more likely that the coats were rendered more permeable to water or gases or both. Outer coats of acid-treated cormils were brittle, indicating that their nature had been considerably changed.

(iv) *Length of Storage at Room Temperature*

One possible explanation for sprouting of some of the cormils collected on March 11, 1960 is that some sort of after-ripening occurred in the interval between collecting and sowing. To check this, batches of 100 cormils, which had been stored air-dry in a closed dark bottle in the laboratory, were dissected at monthly intervals over a 4-month period and the percentage of cormils with enlarged buds was noted. The percentage of cormils showing bud enlargement did not increase with length of storage. In some cases, however, marked bud enlargement took place so that the bud projected through the outer coat. This enlargement only occurred if the cormils had thin, poorly lignified coats, suggesting that physical factors were involved in

cormil dormancy. Cormils with enlarged buds developed rapidly if placed in a moist medium at 25°C.

(v) *Uptake of Oxygen, Vital Stains, and Water*

(1) *Oxygen Uptake.*—An experiment was carried out to determine whether dissected cormils exhibited a higher rate of oxygen uptake than intact cormils. Batches of 20 cormils (intact or dissected completely) were placed in Warburg flasks containing a filter paper and 0·2 ml distilled water. Each centre-well contained 0·2 ml 10% KOH solution. Oxygen uptake readings were made at intervals with a Warburg respirometer (not shaken) over a 3-day period. Very little oxygen uptake was recorded apart from that taken up by dissected cormils on the third day when sprouting commenced.

(2) *Uptake of Vital Stains.*—Intact and dissected cormils were immersed in solutions of water-saturated methylene blue and neutral red for varying periods of time in an attempt to determine how water uptake occurs. Dye penetrated the outer fibrous coat of cormils either through scar tissue at the base of the cormil or through the segmented coat above the terminal bud. Although the inner coat of each cormil stained deeply after several days in the dyes the cormil proper did not, suggesting that the inner coat was impervious to water. If the inner coat was ruptured, buds stained deeply when sprouting commenced.

(3) *Water Uptake.*—Experiments were carried out with intact and dissected cormils to determine water uptake–time patterns for cormils placed in moist conditions in darkness at 25°C. Uptake measurements were made, after cormils had been blotted, on a torsion balance which gave results to the nearest 0·5 mg. A rapid initial uptake of water occurred when an air-dry cormil was moistened, but further uptake ceased unless the cormil sprouted. If this occurred, water uptake followed an exponential curve over the next 14 days. It is suggested that the initial phase of uptake was due to the movement of water into space between inner and outer cormil coats.

These experiments show that rapid and continued uptake of oxygen, dyes, and water occurred only if the inner cormil coat had been ruptured and the bud was enlarging.

(e) *Influence of External Conditions on Dormancy*

(i) *Storage Temperature*

Cormils collected on July 16, 1960 were stored air-dry at 4–5, 20, 25, or 30°C for from 32 to 57 days, after which observations on bud enlargement were made. None of the cormils sprouted in these tests and it is concluded that storage temperature is not an important factor in cormil dormancy.

(ii) *Light Treatments*

Toole *et al.* (1956) state that light treatments may stimulate, retard, or have no effect on seed germination. Since light might also be necessary for the sprouting of cormils, their sprouting behaviour in red and white light was compared with that in darkness. Intact and dissected cormils (inner and outer coats removed) were placed

under moist conditions at 25°C in continuous red light, continuous white light (fluorescent), and darkness. Sprouted cormils were counted 31 days later.

It is evident from the following tabulation that light treatments did not markedly influence the sprouting of cormils, but, in all light regimes, a high percentage of dissected cormils sprouted:

Light treatment	Red		White		Darkness	
Light intensity (f.c.)	0*		800		0	
Dissection treatment	Intact	Dissected	Intact	Dissected	Intact	Dissected
Percentage cormils sprouted	5·0	65·0	7·5	60·0	0	55·0

* No reading with electroselenium-type light-meter.

III. Discussion

Results show that cormil dormancy in Cape tulip is not due to a high level of endogenous growth inhibitors but is due to physical factors associated with the presence of intact cormil coats. Thus the negative results obtained with dormancy-breaking chemicals, leaching tests with water, and bioassays for endogenous growth regulators rule out the importance of chemical factors in cormil dormancy, whereas the success of acid treatment and dissection experiments indicate the importance of physical factors.

Dissection experiments showed that cormil dormancy is due to an intact inner coat and tests with vital stains suggest that this coat is impermeable to water. The inner cormil coat contains both cutin and fat globules, and such a coat would prevent desiccation of inner cormil tissues during hot, dry summers. The lignified outer coat is also obviously involved in cormil dormancy since it protects the thin, papery inner coat. It is significant that those cormils which possessed thin, poorly lignified outer coats readily sprouted, and it is probable that the inner coats of these cormils ruptured during the initial washing, surface-sterilizing, and drying operations in the laboratory.

In the field, cormils would remain dormant until some agency such as cultivation, fire, or action of soil microorganisms causes the breakdown of cormil coats. According to Parsons (1958), long-term dormancy of Cape tulip can be overcome by keeping infested areas bare of vegetation during the summer so that the soil is fully exposed to the sun, or, alternatively, by burning-off surface cover in autumn or cultivation in autumn when the corms are dormant. Such treatments would be expected to overcome cormil dormancy as well and it is suggested that long-term dormancy of Cape tulip corms may also be due to the presence of intact inner and outer coats. However, observations at Yatala Vale showed that a high proportion of cormils were dormant at a time when virtually no corms were dormant, i.e. cormil dormancy may be more persistent and difficult to overcome than corm dormancy. If this is so, it is probably the result of differences in size or differences in the nature of the outer coat in each case or both. The larger size of the corms and their reticulate outer coat may render them more likely to be influenced by a field treatment such as cultivation than cormils.

IV. Acknowledgments

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