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Dormancy in Bulbils of Several Herbaceous Plants: Effects of Photoperiod, Light, Temperature, Oxygen and Gibberellic Acid

NOBUO OKAGAMI

Biological Institute, Faculty of Science, Tohoku University, Sendai 980

Effects of some environmental conditions (photoperiod, white and colored lights, temperature, partial oxygen pressure) and growth regulators (gibberellic acid, 2-chloroethyltrimethylammonium chloride) on induction and release of dormancy of the bulbils of *Dioscorea batatas*, *Laportea bulbifera*, *Elatostema involucratum* and *Sedum bulbiferum* were investigated.

Bulbils were formed under short-day conditions in *Laportea* and *Elatostema*, under long-day conditions in *Sedum*, and irrespective of photoperiods in *Dioscorea*. In all species except *Sedum*, immature bulbils required light, particularly blue or far red, for sprouting (photo-sprouting stage), and mature bulbils required a cold treatment (thermo-sprouting stage). The duration of photo-sprouting and thermo-sprouting stages and the degree of dependency on light or low temperature of sprouting differed from species to species. Sprouting of chilled mature bulbils of these species was promoted by light, especially by red or green light.

Both immature and mature bulbils of *Sedum* sprouted under short-day conditions. Continuous irradiation with blue, far-red and green light markedly inhibited their sprouting.

Oxygen at high concentrations inhibited the sprouting of immature bulbils in *Dioscorea*; in the other species it promoted sprouting regardless of the maturation of the bulbils.

Application of gibberellic acid caused the sprouting of bulbils in the absence of light, chilling or photoperiodic treatment in all species except *Dioscorea*, in which gibberellic acid inhibited sprouting. Polyphenol oxidase activity was very high in the homogenates of *Dioscorea* bulbils, and increased further when the bulbils had been treated with gibberellic acid. In the other species, little or no such activity was observed.

Many herbaceous plants form bulbils (Luftknölchen, in German) in their superterrene shoots. These bulbils are considered to be useful materials for studying bud dormancy, since those detached at various stages of development can be cultured easily, as compared with dormant buds of woody species, subterranean dormant organs of herbaceous species and asexual dispersal organs of aquatic species. Most information on the dormancy of bulbils has been obtained for *Begonia evansiana* (Esashi and Nagao, 1958, 1959, 1973; Nagao and Mitsui, 1959; Esashi, 1962, 1969; Nagao and Okagami, 1966; Cho, 1970a, b, c; Sano and Nagao, 1970; Okagami, 1972; Okagami and Esashi, 1972; Okagami and Nagao, 1973; Okagami *et al.*, 1977), and only meager

Abbreviations: GA_3, gibberellic acid; CCC, 2-chloroethyltrimethylammonium chloride; SD, short day; LD long day.

knowledge is available for other plants.

In the present work, the dormancy of bulbils in several species was investigated from various aspects to obtain more information on bulbil dormancy. A part of the results obtained in this work has been briefly reported in previous papers (Okagami, 1967; Okagami and Nagao, 1971).

Materials and Methods

Plant materials

The species used were *Dioscorea batatas* Decne., *Laportea bulbifera* (Sieb. et Zucc.) Weddel, *Elatostema involucratum* (Franch. et Savat.) Makino, and *Sedum bulbiferum* Makino. *Begonia evansiana* Andr. was used in one experiment. Bulbils of *Laportea* and *Elatostema* were collected during September to October from plants growing spontaneously in the Botanical Garden of Tohoku University (Sendai). Bulbils of *Dioscorea* and *Begonia* were harvested during August to October from plants grown under natural conditions in a nursery in Sendai. Bulbils of *Sedum* were collected in June and July from plants growing spontaneously in fields near our laboratory. Photographs of these bulbils are presented in Fig. 1. In the present paper, length of transversal axis was used as "width" for index of size of bulbils.

For the experiments on bulbil formation, mature bulbils of *Laportea* and *Elatostema* were chilled (2 C) to break dormancy, planted in clay pots, and grown under continuous

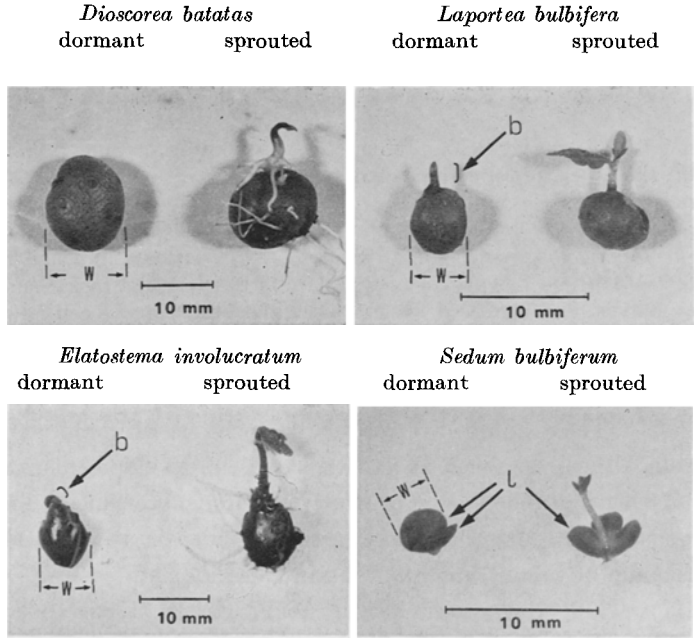

Fig. 1. Dormant and sprouted bulbils of *Dioscorea batatas*, *Laportea bulbifera*, *Elatostema involucratum* and *Sedum bulbiferum*. Dormant bulbils of *Laportea* and *Elatostema* have buds (b) protruding from bulbous portion. *Sedum* bulbils usually have two leaves (l) and sometimes three to four. Transverse axis length was used for index of size of bulbils as width (w).

light (the natural daylight supplemented by fluorescent light) at 15–25 C for about 4 months. Just sprouted young plants of *Sedum* were collected in the fields in December and grown in clay pots under 9-hr SDs (light from fluorescent lamps) at 15–25 C for about 4 months. Then these vegetatively grown plants were subjected to conditions for the bulbil formation.

For the experiments on sprouting, the bulbils (40–70) were incubated on absorbent cotton moistened with distilled water or with aqueous solutions of the chemicals to be tested in 6- or 9-cm Petri dishes.

Light and oxygen

White light of 2.1 W/m^2 (1500 lux, measured by selenium photocell Type–5, Toshiba, Tokyo) was obtained from fluorescent lamps (40-D-SDL, Toshiba), and colored lights (0.5 W/m^2) from the apparatus described previously (Okagami and Kawai, 1977). Energy of irradiance was measured using Kipp & Zonen (Utrecht, Holland) thermopile fitted with fluoride window glass to cut off infrared radiation.

Various oxygen concentrations in nitrogen were obtained by the same methods described previously (Okagami, 1972).

Polyphenol oxidase

Polyphenol oxidase activity was determined in the same way as described before (Okagami, 1972). Homogenates of bulbils in phosphate buffer were used as enzyme preparations. Enzyme activity was measured by oxygen consumption with a Warburg manometer, using hydroquinone as a substrate.

Experiments were repeated at least three times using the bulbils harvested in different years. Consitent trends were observed in the results of these experiments.

Results

Photoperiodic conditions required for bulbil formation

Four-month-old potted plants of *Laportea*, *Elatostema* and *Sedum* were subjected to 9-hr SDs or to continuous light. The SD treatment was given by exposing them to white light obtained from fluorescent lamps at 25 C in a growth chamber in *Elatostema* and *Sedum*, and by covering the plants with light-tight black cloths from 18:00 to 09:00 at 23–30 C in a greenhouse under natural daylight conditions in *Laportea*. Continuous illumination was given by white fluorescent lamps in a growth chamber for *Elatostema* and *Sedum*, and by the natural daylight supplemented by white fluorescent lamps at night in a greenhouse for *Laportea*. As shown in Table 1, *Laportea* and *Elatostema* formed bulbils only under SDs; whereas *Sedum* formed them only under LDs. Further experiments revealed that the critical daylength for bulbil formation in *Laportea* and *Elatostema* was 13–15 hr and the optimum was about 9 hr (Okagami, 1967). In *Sedum*, the optimum daylength was 21–24 hr.

In *D. batatas*, Nakano and Kinoshita (1942) observed that bulbil formation was induced by the hanging down of its vine. Sawada and Yakuwa (1955) reported that

Table 1. Bulbil formation in *Laportea*, *Elatostema* and *Sedum* under 9-hr SDs or continuous light[1)]

Species	Number of bulbils formed/Number of plants tested	
	9-hr	Continuous light
Laportea	93/6	0/12
Elatostema	90/6	0/6
Sedum	0/11	52/11

[1)] Four-month-old plants were subjected to 9-hr SDs or continuous light for 24 days in *Laportea* and *Elatostema* and for 14 days in *Sedum*.

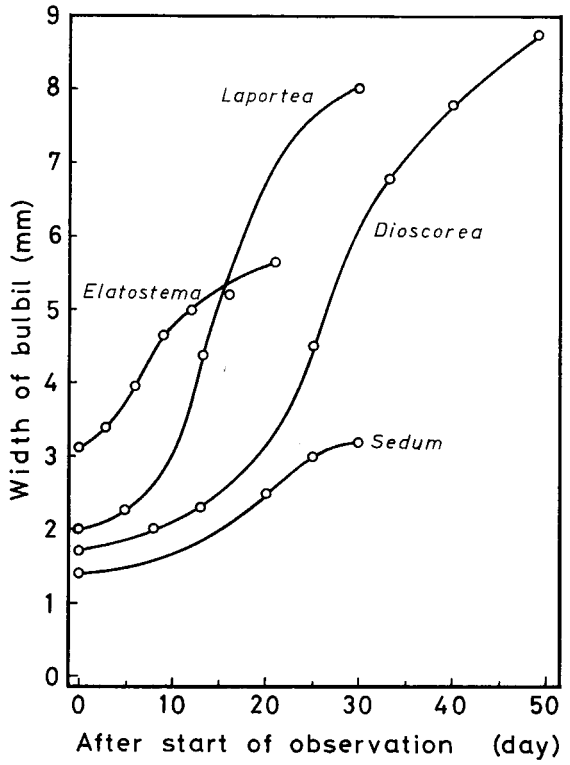

Fig. 2. Maturation process of bulbils of *Dioscorea*, *Laportea*, *Elatostema* and *Sedum*. In *Dioscorea* and *Sedum*, observation was performed on the plants growing in natural conditions. In *Laportea* and *Elatostema*, 4-month-old potted plants were subjected to artificial SDs (9-hr photoperiod). Dates of the start of the observation: August 8, 1967 in *Dioscorea*; June 11, 1967 in *Sedum*; May 25, 1967 in *Laportea*; November 14, 1966 in *Elatostema*. Before start of the observation, *Laportea* and *Elatostema* accepted 10 and 12 SDs, respectively.

bulbil formation took place irrespective of photoperiodic conditions. Their observations were confirmed in the present work (data not shown).

With the progress of maturation, the bulbils thickened and increased their width (Fig. 2). Growth curves of bulbils of all species were sigmoidal.

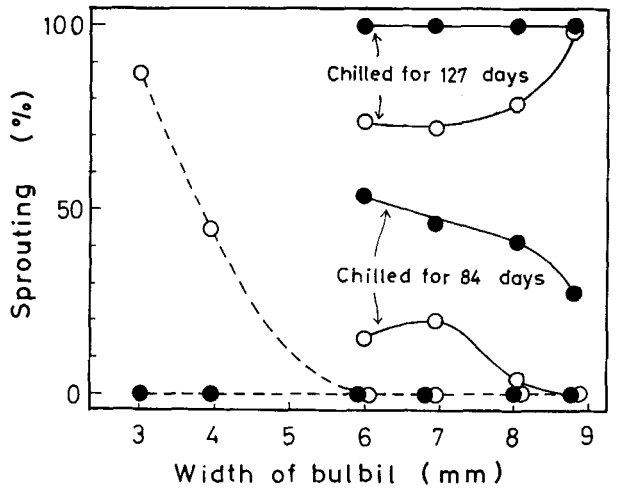

Fig. 3. Changes in the sprouting ability of *Dioscorea* bulbils during their maturation. Bulbils at different maturation stages were incubated at 26 C in light (open circles) or in dark (closed circles) after chilling at 5 C in dark for 84 or 127 days (solid lines) or without chilling (broken lines). Sprouting was observed 170 days after the start of the experiment.

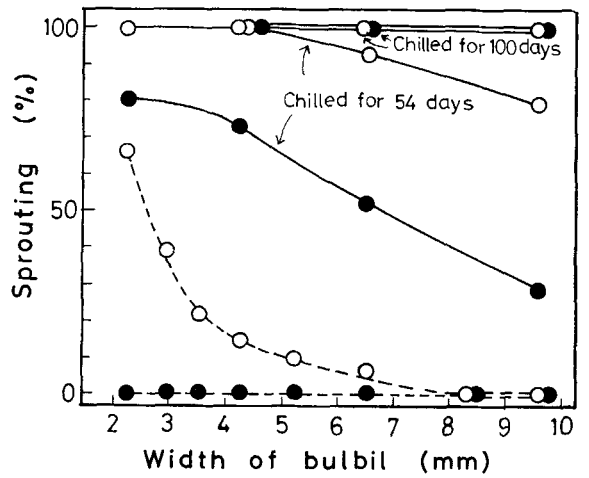

Fig. 4. Changes in the sprouting ability of *Laportea* bulbils during their maturation. Bulbils at different maturation stages were incubated at 28 C in light (open circles) or in dark (closed circles) after chilling at 5 C in dark for 54 or 100 days (solid lines) or without chilling (broken lines). Sprouting was observed 116 days after the start of the experiment.

Environmental conditions required for the sprouting of bulbils at different maturation stages

Bulbils at different stages of maturation were obtained by isolating them from the plants every several days. They were incubated at 24, 26 or 28 C in light or in dark immediately after harvest or after being subjected to a chilling treatment.

Sprouting of unchilled bulbils of Dioscorea, Laportea *and* Elatostema (Figs. 3, 4, 5).

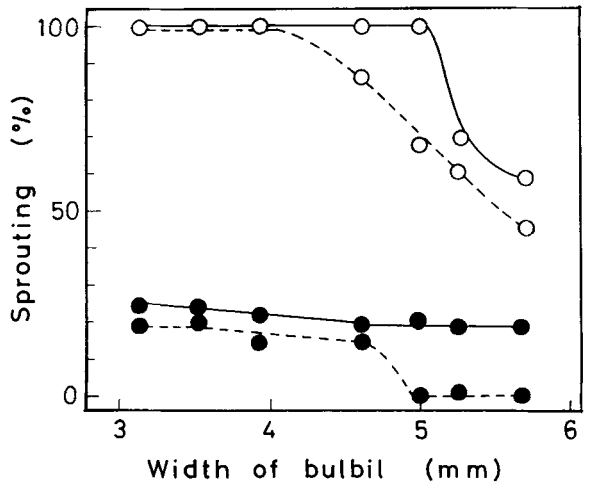

Fig. 5. Changes in the sprouting ability of *Elatostema* bulbils during their maturation. Bulbils at different maturation stages were incubated at 28 C in light (open circles) or in dark (closed circles) after chilling at 5 C in dark for 30 days (solid lines) or without chilling (borken lines). Sprouting was observed 60 days after the start of the experiment.

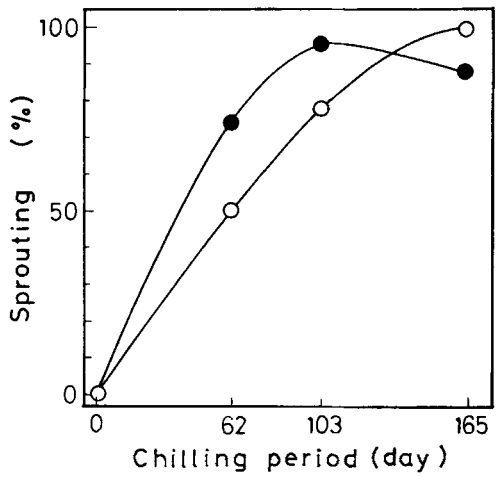

Fig. 6. Sprouting of mature bulbils of *Dioscorea* incubated at 28 C in light (open circles) and in dark (closed cricles) after exposure for various durations to chilling at 5 C in dark. Sprouting was observed 175 days after the start of the experiment.

In dark, none of the unchilled bulbils sprouted at any stage of maturation, except some of the immature bubils of *Elatostema*. In light, however, sprouting occurred considerably in immature bulbils but decreased with the progress of maturation. The length of the immature period during which bulbils could sprout in light differed from species to species. It was longest in *Elatostema*, and *Laportea* and *Dioscorea* followed in this order. The bulbils of *Elatostema* showed about 50% sprouting even after they matured.

Sprouting of chilled bulbils of Dioscorea, Laportea *and* Elatostema. Excepting the immature bulbils of *Dioscorea* which died during chilling, the chilled bulbils of

Laportea (Fig. 4), *Elatostema* (Fig. 5) and *Dioscorea* (Fig. 3) sprouted both in dark and in light, at any stage of maturation. The bulbils of *Dioscorea* became resistant to low temperature when they lost their ability to sprout in response to light with the increase of width. As the bulbils matured, the sprouting ability decreased in the bulbils exposed to a short-term chilling and increased (*Dioscorea*) or remained unchanged (*Laportea* and *Elatostema*) in the bulbils given a long-term chilling (Figs. 3, 4, 5). These results suggest that dormancy in these bulbils deepens with their maturation, and that full-grown *Dioscorea* bulbils exhibit the greatest sprouting ability if their dormancy is broken by sufficient chilling.

In the next experiment, mature bulbils were chilled for various periods, and then incubated in light or dark. The sprouting of *Dioscorea* bulbils given a short-term chilling was inhibited by light exposure during incubation; the inhibitory effect of light decreased with increasing duration of chilling; no inhibition or a slight promotion of sprouting was evident in the bulbils chilled for a sufficiently long period (Fig. 6). In *Laportea* and *Elatostema*, light had a promotive effect on the sprouting of their bulbils regardless of the duration of chilling (Fig. 7).

Next, the effect of chilling temperature on the sprouting of mature bulbils was investigated. The dormant mature bulbils of *Dioscorea*, *Laportea* and *Elatostema* were chilled at temperature ranging from 2 to 14 C for 40 days, and then kept in light at 28 C. As shown in Fig. 8, the optimum temperature for breaking dormancy was 5 C in *Dioscorea* and 2 C in *Laportea* and *Elatostema*.

In *Elatostema*, normal elongation of sprouts was observed in the bulbils chilled for 30 days; but the bulbils of *Laportea* and *Dioscorea* required much longer chilling for normal shoot elongation, producing dwarf plants when the chilling period was insufficient.

Sprouting of bulbils of Sedum. The previous report (Okagami, 1967) suggested that the bulbils of *Sedum* require SDs for sprouting. In the present experiments, immature and mature bulbils were incubated under various photoperiods for 21 days with or without a following incubation in dark for 34 days (Fig. 9). At the end of photoperiodic treatment, a maximum sprouting in immature bulbils was obtained under about an 8-hr photoperiod, the sprouting decreased rapidly with an increase in the daylength. Almost all the immature bulbils sprouted irrespective of the photoperiod, when incubated in dark after the photoperiodic treatment. None of the mature bulbils sprouted at the end of the photoperiodic treatment under any of the photoperiods tested. When the mature bulbils were incubated in dark after the photoperiodic treatment, sprouting occurred to some degree; maximum sprouting was elicited by approximately 8-hr photoperiods. Additional groups of the bulbils were exposed to schedules of 4-hr light – 20-hr dark period interrupted with 1-hr light in the middle of the dark period (light-break). Sprouting was markedly inhibited by the light-break. Sprouting percentages of the bulbils subjected to the light-break correspond to that given a 12.8-hr photoperiod in immature bulbils and to that given a 13.9-hr photoperiod in mature bulbils. These results reveal the involvement of photoperiodic response in sprouting of the

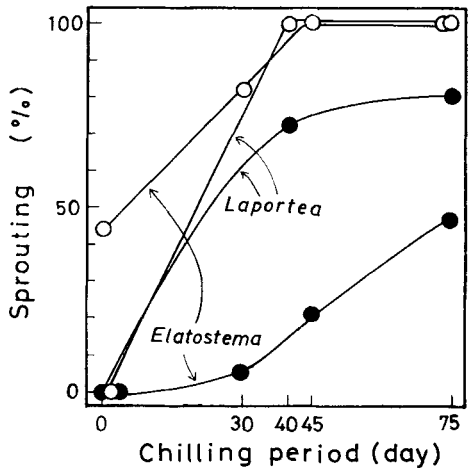

Fig. 7

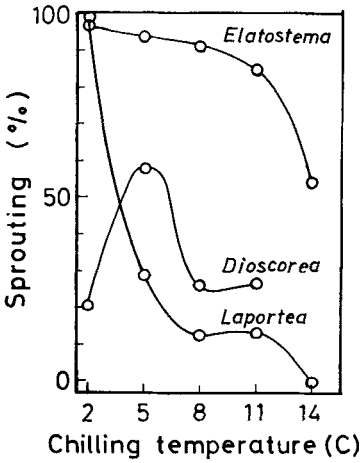

Fig. 8

Fig. 7. Sprouting of mature bulbils of *Laportea* and *Elatostema* incubated in light (open circles) or in dark (closed circles) at 28 C after exposure to chilling at 2 C in dark for various durations. Sprouting was observed 100 days after the start of the experiment.

Fig. 8. Effect of chilling temperature on the sprouting of mature bulbils of *Dioscorea*, *Laportea* and *Elatostema*. After being chilled at various temperature in dark for 40 days, the bulbils were incubated at 28 C for 20 days in light.

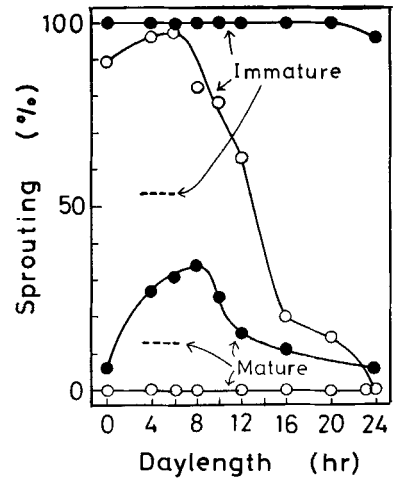

Fig. 9

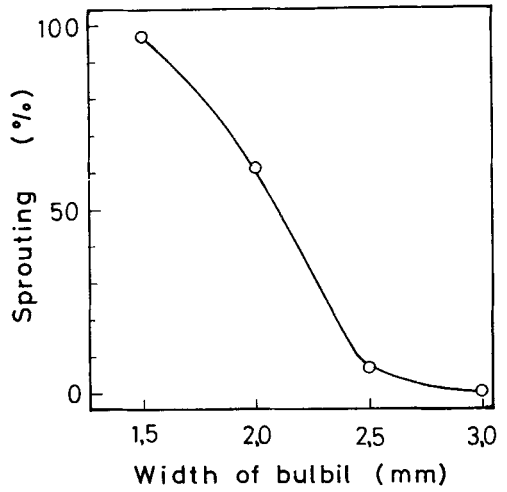

Fig. 10

Fig. 9. Effect of photoperiod on the sprouting of immature and mature bulbils of *Sedum*. Immature and mature bulbils, mean diameter of their leaves were 1.5 and 3.0mm, respectively, were incubated at 24 C under various photoperiods for 21 days. In one group (open circles), sprouting was observed at the end of the photoperiodic treatments, but in other group (closed circles) sprouting was observed after further incubation in dark for 34 days. Horizontal broken lines indicate the sprouting percentages of bulbils exposed to schedules of 4-hr light/20-hr dark interrupted at the middle by 1-hr light for 21 days. Sprouting in immature bulbils was observed at the end of the photoperiodic treatment, and that in mature ones after subsequent 34 days' incubation in dark.

Fig. 10. Changes in the sprouting ability of *Sedum* bulbils during their maturation. Bulbils at different maturation stages were incubated under 9-hr photoperiods at 24 C for 21 days.

bulbils to SDs.

Fig. 10 shows the sprouting ability of bulbils under 9-hr photoperiod at various stages of maturation. The sprouting of bulbils decreased with progress of their maturation.

Effects of colored lights on sprouting

As mentioned before (Figs. 3, 4, 5), the immature bulbils of *Dioscorea*, *Laportea* and *Elatostema* generally required light for their sprouting; and the sprouting of chilled mature bulbils was promoted by light except in the case of *Dioscorea* bulbils exposed to insufficient chilling (Figs. 3, 6). In the present experiments, the effects of colored light on the sprouting of mature and immature bulbils were examined.

Immature bulbils (Table 2). The sprouting of *Dioscorea*, *Laportea* and *Elatostema* bulbils was greatly promoted by blue light. Far-red light also had a promotive effect in *Dioscorea* and *Elatostema*, but not in *Laportea*. Red light promoted the sprouting to a certain extent in *Elatostema* and *Laportea*. Green light slightly promoted the sprouting in *Laportea*. In contrast, the sprouting of *Sedum* was inhibited by blue, far-red and green light, and slightly by red light.

Table 2. Effects of colored light on the sprouting of immature bulbils of *Dioscorea*, *Laportea*, *Elatostema* and *Sedum*[1)]

Light	Sprouting (%)			
	Dioscorea	*Laportea*	*Elatostema*	*Sedum*
Blue	58	50	75	16
Green	37	9	—	26
Red	45	11	47	64
Far red	63	0	82	5
Dark	41	0	20	84

[1)] Immature bulbils of *Dioscorea* were incubated at 28 C for 37 days under continuous irradiation with colored light and then for 25 days in white light. Immature bulbils of *Laportea*, *Elatostema* and *Sedum* were incubated under continuous irradiation with colored light for 90 days at 28 C, 60 days at 28 C and 32 days at 26 C, respectively.

Mature bulbils (Table 3). In *Dioscorea*, *Laportea* and *Elatostema*, sprouting of chilled mature bulbils was greatly promoted by green and red lights. Far-red light also slightly enhanced sprouting in *Laportea* and *Elatostema*. In *Elatostema*, blue was also somewhat effective. The sprouting of *Sedum* mature bulbils (unchilled) was inhibited strongly by far-red, blue and green and slightly by red lights similar to immature ones.

Effect of oxygen concentration on sprouting

Immature bulbils. The immature bulbils of *Dioscorea*, *Laportea* and *Elatostema* were allowed to sprout in light at various oxygen concentrations and those of *Sedum* in dark (Fig. 11). The sprouting of *Dioscorea* bulbils was accelerated in a low-oxygen atmosphere, as in the case of *Begonia* bulbils (Okagami, 1972; Esashi and Nagao, 1973;

Table 3. Effect of colored light on the sprouting of mature bulbils of *Dioscorea*, *Laportea*, *Elatostema* and *Sedum*[1)]

Light	Sprouting (%)			
	Dioscorea	*Laportea*	*Elatostema*	*Sedum*
Blue	68	43	46	0
Green	91	79	66	0
Red	91	71	79	17
Far red	62	56	50	0
Dark	71	40	33	25

[1)] Mature bulbils of *Dioscorea*, *Laportea* and *Elatostema* were chilled in dark at 5 C for 150, 125 and 20 days, respectively, and then incubated under continuous irradiation with colored light or in dark at 28 C for 27, 24 and 30 days, respectively. Mature bulbils of *Sedum* were incubated at 26 C for 32 days without chilling.

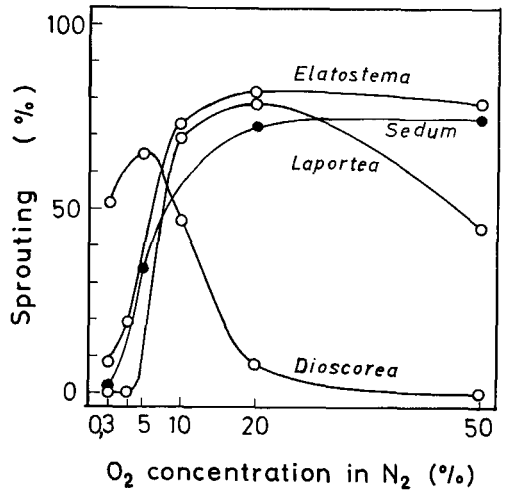

Fig. 11

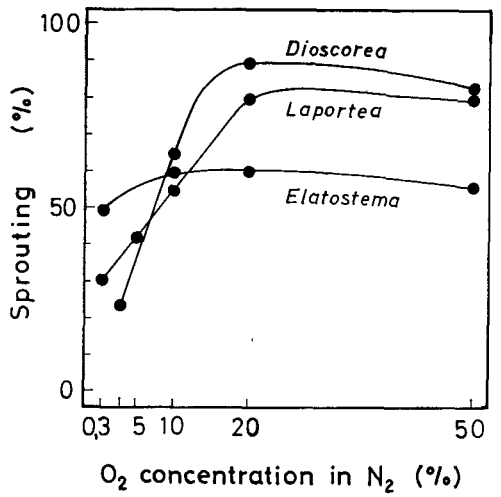

Fig. 12

Fig. 11. Effect of oxygen concentrations on the sprouting of immature bulbils. Bulbils of *Dioscorea*, *Laportea* and *Elatostema* were incubated in light for 42, 70 and 37 days, respectively. *Sedum* bulbils were incubated in dark for 60 days.

Fig. 12. Effect of oxygen concentration during chilling treatment on the sprouting of mature bulbils. Bulbils of *Dioscorea*, *Laportea* and *Elatostema* were chilled at 5 C in various oxygen concentrations in dark for 90, 50 and 30 days, and then incubated in air at 28 C in dark for 18, 12 and 60 days, respectively.

Okagami and Nagao, 1973). In contrast, the bulbils of *Laportea* and *Elatostema* required higher oxygen tensions for sprouting, and a few of them sprouted under lower (0.5–5%) oxygen tensions. No sprouting was caused in the above 3 species under any oxygen tension tested in dark. The sprouting of *Sedum* bulbils was promoted in higher oxygen tensions in dark.

Mature bulbils. The mature bulbils of *Dioscorea*, *Laportea* and *Elatostema* were chilled at 5 C in dark under various oxygen tensions and then incubated in the air at

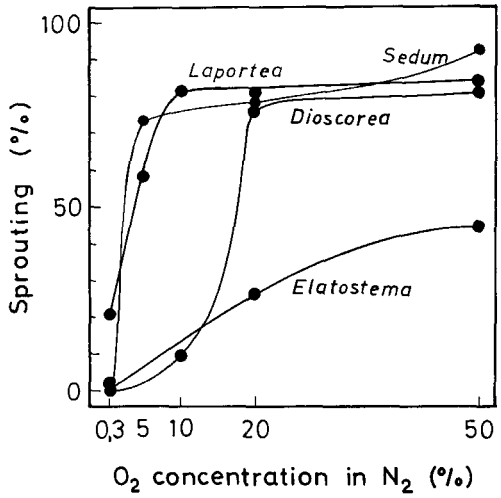

Fig. 13

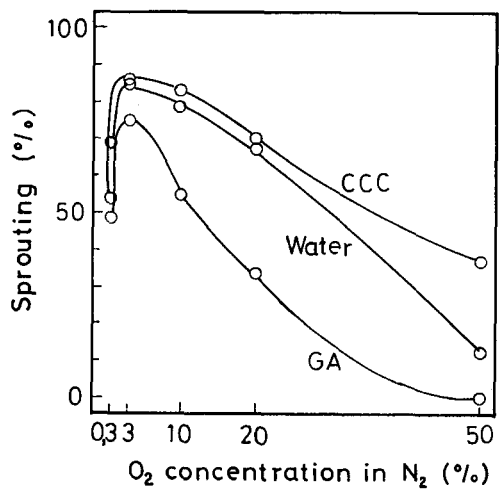

Fig. 14

Fig. 13. Effect of oxygen concentration on the sprouting of mature bulbils. Mature bulbils of *Dioscorea*, *Laportea* and *Elatostema* were chilled in air at 5 C in dark for 200, 80 and 60 days, respectively. Then these bulbils and unchilled mature bulbils of *Sedum* were incubated in various oxygen concentrations at 28 C in dark for 12 days in *Dioscorea*, 25 days in *Laportea*, 30 days in *Elatostema* and 75 days in *Sedum*.

Fig. 14. Effects of GA_3 and CCC on the sprouting of immature bulbils of *Dioscorea* in various oxygen concentrations. Immature bulbils were incubated in light at 28 C with 0.6 μM GA_3 or 3 mM CCC in various concentrations of oxygen for 33 days.

28 C in dark (Fig. 12). The chilling treatment under lower oxygen concentration reduced the sprouting activity of *Dioscorea* and *Laportea* bulbils, as compared with that in normal oxygen tension (20%), but the sprouting of *Elatostema* bulbils was hardly affected by oxygen concentrations during chilling, which caused about 50% sprouting irrespective of oxygen concentrations.

In the next experiment, the mature bulbils of the above 3 species were chilled in the air at 5 C in dark. These bulbils and unchilled mature bulbils of *Sedum* were incubated in various oxygen tensions at 28 C in dark (Fig. 13). In all species, sprouting of mature bulbils increased with increasing concentration of oxygen.

Effects of GA_3 and CCC on Sprouting

Dioscorea. As previously reported (Okagami and Nagao, 1971; Okagami and Tanno, 1977; Okagami, 1978), dormancy of *Dioscorea* bulbils is induced by GA_3. In the present experiment, the effects of GA_3 and CCC were examined in relation to oxygen tensions. The immature bulbils of *Dioscorea* were incubated in light at 28 C with or without 0.6 μM GA_3 or 3 mM CCC under various oxygen tensions for 33 days (Fig. 14). As observed before (Fig. 11), the sprouting of bulbils incubated in water was inhibited by high concentrations of oxygen. Inhibition of the sprouting by GA_3 was more remarkable in higher concentrations of oxygen. CCC reduced the inhibition caused by higher concentrations of oxygen.

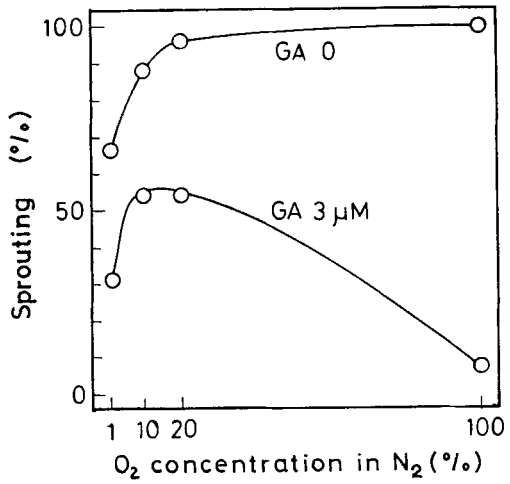

Fig. 15. Effect of GA_3 on the sprouting of chilled mature bulbils of *Dioscorea* under various oxygen concentrations. Mature bulbils were incubated with GA_3 in light at 28 C for 8 days after being chilled at 5 C in dark for about 6 months.

Next, the mature *Dioscorea* bulbils chilled at 5 C in air for 6 months were incubated in light with or without 3 μM GA_3 under various oxygen tensions (Fig. 15). The inhibitory effect of GA_3 on sprouting was enhanced with increasing concentration of oxygen, and practically no bulbils sprouted in 100% oxygen.

Laportea, Elatostema *and* Sedum. The immature bulbils of *Laportea* and *Elatostema* were incubated with or without GA_3 in light or in dark (Table 4). The sprouting of these bulbils was stimulated by GA_3, which inhibited sprouting in *Dioscorea* (Figs. 14, 15). It is interesting that the immature bulbils which required light irradiation for sprouting (see also Figs. 4, 5) sprouted even in dark in the presence of GA_3.

The effect of GA_3 on the sprouting of dormant mature bulbils of *Laportea*, *Elatostema* and *Sedum*, which received no chilling, are presented in Fig. 16. GA_3 promoted the sprouting of these bulbils regardless of light conditions during incubation. Sprouting increased with increasing concentrations of GA_3. *Elatostema* bulbils were particularly sensitive to GA_3.

Table 4. Effect of GA_3 treatment on the sprouting of immature bulbils of *Laportea* and *Elatostema* in light and in dark[1)]

Species	GA_3 concentration	Sprouting (%)	
		Light	Dark
Laportea	0	45	0
	30 μM	95	84
Elatostema	0	4	0
	2 μM	100	100

[1)] Immature bulbils of *Laportea* and *Elatostema* were incubated with or without GA_3 in light or in dark for 25 days.

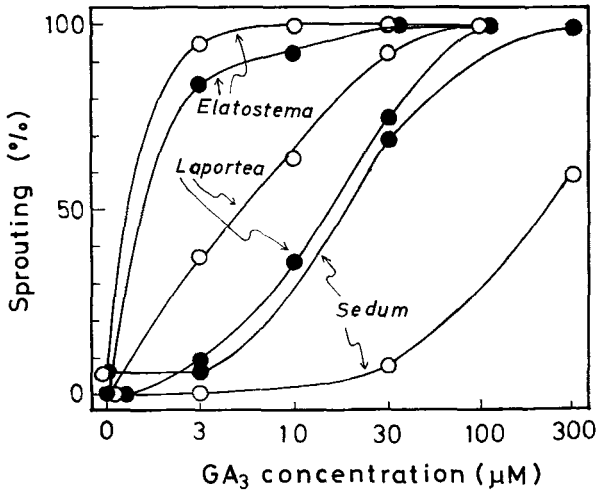

Fig. 16. Effect of GA_3 on the sprouting of dormant mature bulbils of *Laportea*, *Elatostema* and *Sedum*. Unchilled bulbils were incubated with GA_3 at 28 C for 17 days in *Laportea* and *Elatostema* and at 24 C for 60 days in *Sedum*. Open cricles, incubated in light; closed circles, incubated in dark.

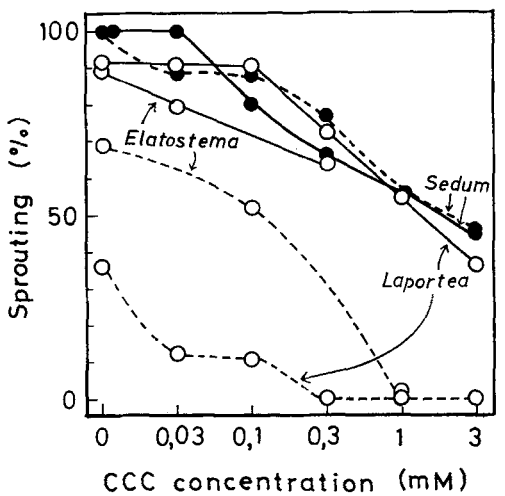

Fig. 17. Effect of CCC on the sprouting of immature (broken lines) and mature (solid lines) bulbils of *Laportea*, *Elatostema* and *Sedum*. Immature and chilled (5 C, 6 months) mature bulbils of *Laportea* were incubated with CCC in light for 55 days. Immature and chilled (5 C, 2 months) mature bulbils of *Elatostema* were incubated with CCC in light at 28 C for 65 and 30 days, respectively. Immature and mature bulbils of *Sedum* were incubated with CCC in dark for 100 days.

Fig. 17 shows the effect of CCC on the sprouting of immature and chilled mature bulbils of *Laportea* and *Elatostema*, and of unchilled mature bulbils of *Sedum*. CCC markedly inhibited the sprouting of these bulbils, while it promoted the sprouting of *Begonia* and *Dioscorea* bulbils (Nagao and Okagami, 1966; Okagami and Nagao, 1971;

Table 5. Polyphenol oxidase activity in homogenates of immature bulbils incubated with or without GA_3[1)]

Species	Polyphenol oxidase activity (O_2 uptake) (μl/hr/g fresh weight of bulbils)	
	No GA_3	GA_3 300 μM
Begonia	255	368
Dioscorea	292	550
Laportea	14	9
Elatostema	Undetected	Undetected
Sedum	Undetected	Undetected

1) Immature bulbils were incubated with or without GA_3 at 28 C in light for 4 days, then homogenized in 0.05 M potassiumphosphate buffer, pH 5.6. Reaction mixture for measurement of polyphenol oxidase consisted of 0.017 M hydroquinone and homogenates of bulbils (0.2 g fresh weight) in 3 m*l* of 0.03 M potassiumphosphate buffer, pH 5.6. Oxygen uptake was measured at 28 C.

Okagami and Tanno, 1977; Okagami *et al.*, 1977; Okagami, 1978).

Polyphenol Oxidase Activity

Polyphenol oxidase, especially the laccase type, is observed to be involved in dormancy induction, particularly in GA_3-induced dormancy, in *Begonia* and *Dioscorea* (Okagami, 1972, 1978). In this experiment, the activity of polyphenol oxidase in the homogenates of immature bulbils of the four species and of *Begonia* was measured. Before homogenization, the bulbils used were incubated with or without GA_3 for 4 days at 28 C in light (Table 5). The homogenates of *Begonia* and *Dioscorea* bulbils showed high polyphenol oxidase activity which increased markedly when the bulbils had been treated with GA_3; whereas those of *Laportea*, *Elatostema* and *Sedum* bulbils exhibited a very low or no activity regardless of GA_3 treatment.

Table 6. Characteristics of dormancy

Species	Condition required for bulbil formation	Condition required for bulbil sprouting		Color of light promotive for sprouting	
		Immature	Mature	Immature	Mature
Dioscorea batatas	Hanging down of vine[1)]	Light	Low temperature	Blue	Red
Bagonia evansiana	SD[3)]	Light[4)]	Low temperature	Blue Far red[5)]	Blue Far red[5)]
Leportea bulbifera	SD	Light	Low temperature	Blue	Red Green
Elatostema involucratum	SD	Light	Low temperature	Blue Far red	Red Green
Sedum bulbiferum	LD	SD	SD		

1) Nakano and Kinoshita, 1942; Sawada and Yakuwa, 1955.
2) Okagami and Nagao, 1971; Okagami and Tanno, 1977; Okagami, 1978.
3) Esashi and Nagao, 1958.
4) Esashi and Nagao, 1959.
5) Esashi, 1969.

Discussion

Some properties of dormancy in the bulbils of four herbaceous plants observed in the present study are summarized in Table 6, together with the results obtained with *Begonia* bulbils (Esashi and Nagao, 1958, 1959, 1973; Nagao and Mitsui, 1959; Esashi, 1962, 1969; Nagao and Okagami, 1966; Cho, 1970c; Okagami, 1972; Okagami and Nagao, 1973; Okagami *et al.*, 1977).

Dormancy development. As briefly reported in the previous paper (Okagami, 1967), the bulbils of *Dioscorea, Laportea* and *Elatostema* required light irradiation for sprouting when immature, and a chilling treatment when mature (Figs. 3–8); that is, these bulbils proceeded from the "photo-sprouting stage" to the "thermo-sprouting stage" during their maturation, and these two stages may correspond to the "summer" and "winter dormancy" (Wareing, 1956) of buds in woody species, respectively, such as *Begonia* bulbils (Esashi and Nagao, 1959; Esashi, 1962). These two stages are also observed in bulbils of 7 species of the genus *Dioscorea* (Okagami and Tanno, 1977). The duration of these two stages and the strictness of light and low-temperature requirements for sprouting differed from species to species. The length of the photo-sprouting stage in *Elatostema* was very long compared to that in *Dioscorea* or *Laportea* (Figs. 3, 4, 5). The range of temperature effective in breaking dormancy of mature bulbils was much wider in *Elatostema* than in *Laportea* and *Dioscorea* (Fig. 8). The mature bulbils of *Elatostema* and *Dioscorea* were capable of sprouting without any chilling treatment after being stored at room temperature (10–25 C) for about 4 and 6–8 months, respectively; while those of *Laportea* failed to sprout during more than 4 years unless chilling treatment was applied. Such differences in temperature-dependency among species is probably related to their ecological habits.

In nature, dispersion by bulbils at the photo-sprouting stage could not be observed. Therefore, the biological significance of the photo-sprouting stage is difficult to

of bulbils in herbaceous plants

Suitable oxygen concentration for sprouting		Effect of GA_3 on sprouting	Effect of CCC on sprouting	Activity of polyphenol oxidase
Immature	Mature			
Low	High	Inhibitive[2)]	Promotive[2)]	High
Low[6)]	High[6)]	Inhibitive[7)]	Promotive[8)]	High
High	High	Promotive	Inhibitive	Low
High	High	Promotive	Inhibitive	Undetected
High	High	Promotive	Inhibitive	Undetected

[6)] Okagami, 1972; Esashi and Nagao, 1973; Okagami and Nagao, 1973.
[7)] Nagao and Mitsui, 1959; Cho, 1970c; Okagami, 1972; Okagami and Esashi, 1972; Okagami *et al.*, 1977
[8)] Nagao and Okagami, 1966; Okagami *et al.*, 1977.

explain. One possibility would be that during evolution the thermo-sprouting ability was acquired after the photo-sprouting ability with extension of the growing region to colder areas. The photo-sprouting stage may be a relic of the past.

The photo-sprouting stage bulbils of *Begonia*, *Dioscorea*, *Laportea* and *Elatostema* contain a certain amount of chloroplast, though it seems that photo-sprouting does not require photosynthesis, at least not a great amount, since DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea], an inhibitor of photosynthesis, did not inhibit the photo-sprouting (data not shown).

The requirement of light for the photo-sprouting of immature bulbils of *Dioscorea*, *Laportea* and *Elatostema* may not be caused by photoperiodism, since neither obvious critical daylength nor light-break effect was observed, and the sprouting percentage increased with the increase of irradiation energy, as in the case of *Begonia* immature bulbils (Esashi, 1962) (data not shown).

LD-Induced dormancy. Plants, such as *Sedum* forming dormant buds under LDs and breaking dormancy under SDs are very rare in the plant kingdom. Under natural conditions in Japan, the bulbils of *Sedum* are formed in June to early July and sprout in November to December. In a preliminary experiment it was observed that life cycle of *Allium nipponicum* Franch. et Savat., a bulbil-forming species in Japan, was regulated by almost the same environmental factors as those of *Sedum*.

Colored light. The most effective color of light required for sprouting in the immature bulbils of *Dioscorea*, *Laportea* and *Elatostema* was blue and far-red or blue alone (Table 2), as in the case of *Begonia* (Esashi, 1962); while these lights strongly inhibited sprouting in *Sedum* (Tables 2, 3). Such a difference between *Sedum* and the other species may be related to a difference in photoperiodic conditions causing the formation of bulbils. In *Begonia* (Esashi and Nagao, 1958), *Laportea* and *Elatostema* (Table 1) bulbils are formed under SDs, whereas in *Sedum*, under LDs (Table 1). In flowering of many LD plants, blue or far-red lights promote the LD action when given as a supplementary light (Stolwijk, 1952; Friend, 1964). The inhibition of sprouting in *Sedum* and the promotion of sprouting in the other species under blue or far-red irradiation may result from the LD action promoted by such light. Although bulbil formation in *Dioscorea* was brought about irrespective of photoperiodic conditions, the sprouting of immature bulbils of this plant was promoted by blue and far-red light (Table 2). Probably, the *Dioscorea* bulbils formed without SD treatment are in a dormant state similar to that of *Laportea*, *Elatostema* and *Begonia* bulbils induced by SDs.

When bulbils of each species used in the present work were irradiated alternatively with red and far-red lights, red/far-red reversibility could not be observed in the sprouting of buds. In *Elatostema* bulbils, however, red/far-red reversibility was observed in rooting so that bulbils of this species may contain phytochrome (data not shown).

In *Begonia* bulbils, red light irradiation inhibits the sprouting (Esashi, 1962, 1969) and causes an increase in the content of the sprouting inhibitor (Okagami *et al.*, 1964). In the other species tested in the present study such red light-induced inhibition

of the sprouting could not be observed (Table 2, 3).

Oxygen. Sprouting promotion under limited oxygen tension in the immature bulbils and under higher tension in the mature ones of *Dioscorea* is similar to the results obtained in *Begonia* (Okagami, 1972; Esashi and Nagao, 1973). In *Laportea*, *Elatostema* and *Sedum*, on the other hand, the sprouting of both immature and mature bulbils occurred under higher oxygen concentrations (Figs. 11–13). Polyphenol oxidase activity is very high in the homogenates of *Dioscorea* and *Begonia* bulbils compared to *Laportea*, *Elatostema* and *Sedum* (Table 5). In *Dioscorea* and *Begonia*, the laccase-type polyphenol oxidase activity increased after GA_3 treatment (Table 5, Okagami, 1972), and GA_3 inhibition of sprouting was released by inhibitors of this enzyme (data not shown). In *Dioscorea*, moreover, increase of activity of this enzyme during incubation is suppressed by protein-synthesis inhibitors such as 8-azaguanine and cycloheximide which promote sprouting (Okagami, 1978). These results support the probable involvement of polyphenol oxidase in dormancy induction. In general, polyphenol oxidase has low affinity for oxygen so that oxygen tension may be a limiting factor for action of this enzyme. Accordingly, sprouting promotion by semi-anaerobiosis is perhaps due to suppression of the activity of polyphenol oxidase.

In 0.3%–5% oxygen in nitrogen, immature bulbils of *Begonia* and *Diosocrea* sprouted to a large extent (Okagami, 1972; Esashi and Nagao, 1973; Figs. 11, 14), while mature bulbils of these species and both immature and mature ones of the other species died or sprouted to a very low extent (Figs. 11–13). These facts suggest that sprouting-inducing metabolisms of immature bulbils of *Begonia* and *Dioscorea* have a high affinity to oxygen.

Growth regulators. It is well known that the dormancy of seeds and buds of many plants is broken by GA_3 (see Stuart and Cathey, 1961). In contrast, bulbil dormancy in *Dioscorea* (Okagami, 1967, 1978; Okagami and Nagao, 1971; Okagami and Tanno, 1977) and *Begonia* (Nagao and Mitsui, 1959; Cho, 1970c; Okagami, 1972; Okagami and Esashi, 1972; Okagami and Nagao, 1973; Okagami *et al.*, 1977) is induced by GA_3. In these species and some other species of the genus *Dioscorea*, endogenous and exogenous gibberellins enhance both dormancy-inducing and sprouting-inducing systems, but they more actively enhance the former system (Okagami, 1972; Okagami and Tanno, 1977). The immature bulbils, at the photo-sprouting stage, of *Laportea* and *Elatostema* could sprout in contact with GA_3 without light irradiation (Table 4), and similarly, the mature bulbils treated with GA_3 could srpout without chilling treatment (Fig. 16). The mature bulbils of *Sedum* could sprout also with GA_3 without SD treatment (Fig. 16). Application of CCC, which inhibits gibberellin biosynthesis (see Lang, 1971), induces the sprouting of bulbils of *Begonia* (Nagao and Okagami, 1966; Okagami *et al.*, 1977) and *Dioscorea* (Okagami and Nagao, 1971; Okagami and Tanno, 1977; Okagami, 1978), but inhibits the sprouting of immature and mature bulbils of *Laportea* and *Elatostema* even in light or after chilling, and *Sedum* bulbils in dark (Fig. 17). These results suggest that not only exogenous GA_3 but also endogenous gibberellin participate as sprouting-inducer in *Laportea*, *Elatostema* and *Sedum* bulbils; in these species gibberellin

may activates more strongly the sprouting-inducing system. In *Laportea* it is of interest, however, that the promotive effect of GA_3 pretreatment on the sprouting of bulbils tends to be enhanced by the subsequent treatment with an inhibitor of polyphenol oxidase (Nagao *et al.*, 1966). This suggests the possibility that the dormancy-inducing system may also be present even in these bulbils and GA_3 activates this system weakly than sprouting-inducing system. Whether gibberellin seemingly acts as a dormancy inducer or a sprouting inducer may be determined by the relative strength of the two gibberellin-activating counteractive systems.

Among bulbils of tested species and *Begonia*, the present study revealed coincident characters that species whose dormancy is induced by gibberellin (*Begonia* and *Dioscorea*) sprout in response to semi-anaerobiosis when immature (Fig. 11; Okagami, 1972; Esashi and Nagao, 1973) and they contain a high activity of polyphenol oxidase (Table 5). These facts also support the assumption that the responsiveness to gibberellin of sprouting of bulbils is based on the activity of polyphenol oxidase. Whether gibberellin seemingly inhibits or promotes the sprouting of bulbils may depend on the activity of polyphenol oxidase. The different strength of effect of GA_3 on the sprouting of several species in the genus *Dioscorea* was observed (Okagami and Tanno, 1977). In these species the stronger the inhibition of the sprouting by GA_3, the higher the enzyme activity (data not shown).

These physiological properties of bulbils obtained in the present work may be found in other similar dormant organs such as winter buds of woody species and various asexual dormant organs of herbaceous species, etc.

More detailed work on several aspects of bulbil dormancy were performed as follows: on the dormancy of bud meristem in *Begonia* (Cho, 1970a, b, c) and *Laportea* (Tanno, 1977); on accumulation of sprouting inhibitors by GA_3 treatment in *Begonia* (Nagao *et al.*, 1966; Okagami and Nagao, 1973), *Dioscorea bulbifera* (Tanno and Okagami, 1974) and *D. batatas* (Hasegawa and Hashimoto, 1974); on the protein synthesis in dormancy induction of *Begonia* (Esashi and Leopold, 1970; Okagami, 1972), *Dioscorea* (Okagami, 1978) and *Laportea* and *Elatostema* (Tanno and Okagami, 1978); on the relation between the increase of the sprouting inhibitor and polyphenol oxidase (Nagao *et al.*, 1966; Okagami and Nagao, 1973).

The experiments in this study were performed with apparatus of the Environmental Control Section of the Biological Institute, Faculty of Science, Tohoku University.

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