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A STUDY ON CERTAIN ASPECTS OF SEED GERMINATION AND GROWTH OF PISTIA STRATIOTES L.

A. H. PIETERSE[1], L. DE LANGE[2] and L. VERHAGEN[2]

[1]Koninklijk Instituut voor de Tropen, Afdeling Agrarisch Onderzoek, Mauritskade 63, 1092 AD Amsterdam
[2]Hugo de Vries Laboratorium, Universiteit van Amsterdam, Sarphatistraat 221, 1018 BX Amsterdam

SUMMARY

The viability of *Pistia stratiotes* L. seeds was not affected by a period of two months in water at 4°C while most seeds could even stand a few weeks in ice at –5°C. Germination did not occur below a temperature of cira 20°C whereas a temperature around 25°C was optimal for germination. There was an after-ripening period of circa 6 weeks. Contradictory to a previous report, the submerged seeds were able to germinate if the light intensity was sufficiently high; seeds did not germinate in the dark. It was concluded that in all probability *Pistia* seeds would be able to survive the Dutch winter season on the bottom of a waterbody. However, the chances for this weed to become permanently integrated in the Dutch flora are considered to be extremely small, in connection with the rarity of seed formation and the adverse conditions for germination prevailing in this country.

In contrast to an earlier report it was observed that growth of *Pistia* on a modified Long-Ashton medium is strongly inhibited at pH 4 while there is a growth optimum at pH 7. As a consequence the conclusions on the ecology of *Pistia* in relation to its pH requirements, which repeatedly turn up in the literature, especially with regard to mixed vegetations with *Eichhornia crassipes* should be reconsidered.

1. INTRODUCTION

Pistia stratiotes L., water lettuce, is a free-floating aquatic plant which occurs in tropical and sub-tropical areas over the whole world. It is generally considered a troublesome aquatic weed since it frequently forms dense vegetations on the surface of waterways and lakes (SCULTHORPE 1967; HOLM et al. 1969, 1977; PIETERSE 1977b). These dense mats may seriously hamper the waterflow, hinder the navigation and provide a favourable habitat for disease carrying mosquitos.

In the summer of 1976 there was an excessive development of *Pistia* in The Netherlands, in the 'Westland', an area south-east of the city of The Hague (DIJKHUIZEN 1976; PIETERSE 1977a; MENNEMA 1977) while the plants were also recorded in the 'Krimpenerwaard', at a distance of circa 40 km from the 'Westland' (MENNEMA 1977). This was very remarkable as the growth habitat of *Pistia* has never been observed to extend into the temperate zones. On the other hand, the Dutch summer of 1976 was extremely hot and the circumstances during the summer months were more or less comparable with tropical conditions. In general it was not assumed that *Pistia* could survive the Dutch winter season although flowering specimens had been collected which produced ripe seeds (PIETERSE 1977a). The explosion of 1976 was explained by a dumping of plants from a tropical aquarium (MENNEMA 1977). This point of view was substantiated by the fact that *Pistia* did not reappear in the 'Westland' during the following summers.

However, *Pistia* had also been recorded in The Netherlands in 1973, although in very small numbers. The sites were in the 'Krimpenerwaard' (QUENÉ-BOTERENBROOD & MENNEMA 1974; VAN SELM 1974) and near the city of Leerdam (MENNEMA & VAN OOSTSTROOM 1974), circa 20 km east of the 'Krimpenerwaard'. Recently, in the summer of 1979, the plant appeared again, this time in relatively large quantities in a canal in the village of Stompwijk (between the cities of Leiden and The Hague).

As a consequence questions were raised about the ecology of *Pistia* in connection with its growth potential in Dutch water bodies and the chances for its survival of the Dutch winter season. However, very few papers about the ecology of *Pistia* have been published and, although the plants themselves do not survive freezing temperatures, the environmental factors which regulate seed germination, as well as the cold-hardiness of the seeds are virtually unknown. The only report on seed germination in *Pistia* refers to the observation (DATTA & BISWAS 1970) that the seeds do not germinate when they are submerged, which implies that germination would be confined to the shoreline.

In the literature there seems to be a contradiction with regard to another ecological aspect, viz. the effect of pH on growth. In water cultures there was a growth optimum at pH 4 when the plants were cultivated on a modified Long-Ashton medium as well as on tap water (CHADWICK & OBEID 1966). However, these extremely low pH values have never been recorded in the field in water-bodies where *Pistia* grows luxuriously. During a study in 1971 and 1972 in lake Volta, an artificial lake in Ghana which in that period was covered by vast vegetations of *Pistia*, the pH was generally around 6.5 and never dropped below 5.7 (HALL & OKALI 1974).

The objectives of the present investigation were to look into the possibilities of *Pistia* seeds surviving the Dutch winter season and germinating under Dutch circumstances, as well as to reinvestigate the germination of *Pistia* plants under submerged conditions and the effect of pH on their growth.

2. MATERIALS AND METHODS

In a heated greenhouse in Amsterdam, a *Pistia stratiotes* L. strain of unknown origin ('greenhouse' strain), which was used as experimental material by PIETERSE (1978), and a strain which was collected in The Netherlands in the 'Westland' area during the outbreak of 1976 ('Westland' strain), were grown in polyethylene reservoirs (55 cm wide, 70 cm long, 50 cm deep). The reservoirs were filled up to the border with tap water and contained a 20 cm thick layer of clay on the bottom. The *Pistia* plants flowered profusely and during the period September 1978–September 1979 seeds were regularly harvested. These seeds were stored under different conditions in environmental chambers in the dark.

The following experiments on seed germination were carried out:

a) Determination of the percentage germinating seeds at different temperatures, i.e. 15°, 17.5°, 20°, 22.5°, 25° and 30°C, after
   1 – wet (submerged) as well as dry storage during $7\frac{1}{2}$ months at 28°C ('greenhouse' strain)
   2 – wet (submerged) storage during one month at 10°C ('greenhouse' and 'Westland' strain).

b) Determination of the percentage germinating seeds at 25°C ('greenhouse' and 'Westland' strain), which after being collected in the greenhouse, were stored in water during various periods of time at respectively 4°C, 10°C and 28°C.

c) Determination of the percentage germinating seeds (at 25°C) after freezing the seeds during various periods of time in ice at –5°C.

d) Determination of the percentage germinating seeds (at 25°C) when submerged in tap water at a depth of respectively 25, 50, 75 and 100 cm under various light intensities.

e) Growth of *Pistia* plants ('greenhouse' strain) at various pH values on
   1 – 1/5 strength Long-Ashton medium (Hewitt, 1952) in an environmental chamber at 25° ± 2°C and
   2 – a combination of 1/5 strength Long-Ashton medium and tap water or half tap/half rain water in the greenhouse.

Experiments a, b and c on seed germination were carried out by placing the seeds in a petri dish with a diameter of 9 cm (20 seeds per dish) containing 3 layers of Whatman no. 1 filter paper wetted by adding 12 ml of distilled water. The petri dishes were kept in environmental chambers at the required temperature under conditions of continuous light of 12.000 erg.cm^{-2}.sec^{-1} intensity from white fluorescent tubes. The number of germinated seeds was assessed after a period of two weeks.

The experiments d) were carried out in 100 cm high, opaque polyethylene pipes (diameter 19 cm), under conditions of continuous light from white fluorescent tubes. The different water depths were obtained either by varying the water level or by having the pipes filled to the brim with water but containing different amounts of clean washed sand. The light intensity at the level of the submerged seeds (at the bottom of the water column) was 4000 erg. cm^{-2}.sec^{-1} in pipes with 25 cm sand, 2000 erg.cm^{-2}.sec^{-1} in pipes with 50 cm sand, 1100 erg.cm^{-2}.sec^{-1} in pipes with 75 cm sand and 900 erg.cm^{-2}.sec^{-1} in pipes without sand. The number of germinated seeds was assessed by counting the number of floating seedlings which appeared at the water surface within a period of four weeks. For each experiment there were two replicates.

The effect of pH on the growth of *Pistia* plants ('greenhouse' strain) was tested in an environmental chamber and in the greenhouse (experiment e). The experiments in the environmental chamber were carried out in polyethylene trays, 45 cm long, 30 cm wide and 8 cm deep, which were filled up to the border with 1/5 strength Long-Ashton medium (CHADWICK & OBEID 1966). The pH was adjusted every day by adding diluted NaOH or HCl. The experiments were started with three *Pistia* plants and after a period of four weeks fresh weight of the plants was determined. The plants were exposed to a continuous illumination of 12.000 erg.cm^{-2}.sec^{-1} from white fluorescent tubes and the temperature was kept at 25°C ± 2°C. Every week the plants were removed to a fresh medium and there were 2 replicates for each pH level. The experiments in the greenhouse were carried out during the summer season in polyethylene reservoirs (55 × 70 × 50 cm). The reservoirs were filled either by tap water or half tap water and half rain water and subsequently supplemented with the macro and micro nutrients of 1/5 strength Long-Ashton medium. The pH was adjusted every day with diluted NaOH and HCl. The experiments were started with five *Pistia* plants and after a period of four weeks fresh weight, dry weight and number of plants was de-

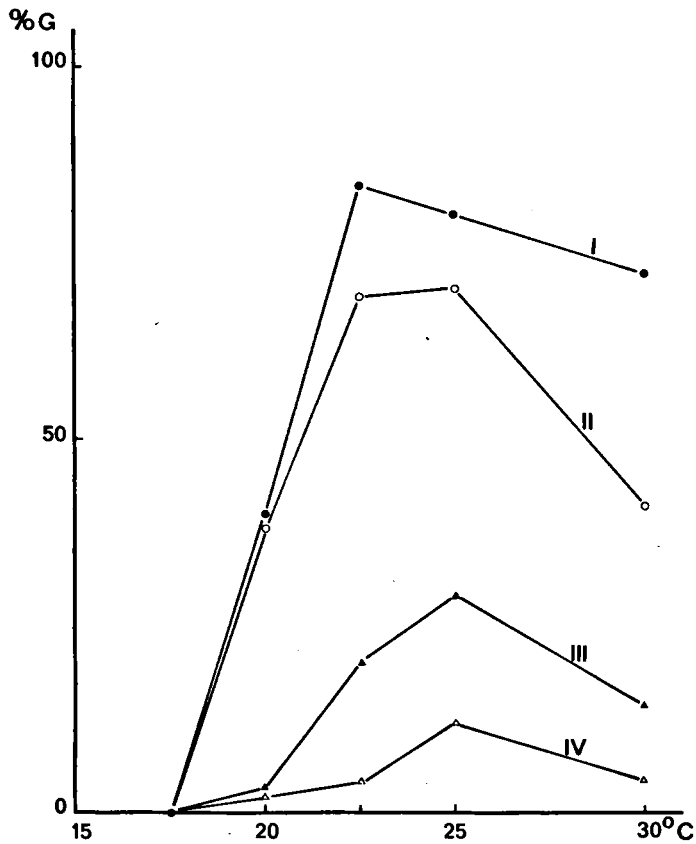

Fig. 1. Percentage of germinating *Pistia stratiotes* seeds (G) at various temperatures. I = 'greenhouse' strain seeds which were stored in water at 28°C and tested 7½ months after shedding. II = 'greenhouse' strain seeds which were stored at 28°C under dry conditions and tested 7½ months after shedding. III = 'greenhouse' strain seeds which had been stored in water at 10°C and tested one month after shedding. IV = 'Westland' strain seeds which had been stored in water at 10°C and tested one month after shedding.

termined. Both in the environmental chamber and the greenhouse the pH was measured with a portable pH meter E488 Metrohm Herisau.

3. RESULTS

The percentage germinating seeds at different temperatures is shown in *fig. 1*. At 15°C and 17.5°C there was no germination of seeds of the 'greenhouse' strain after wet (submerged) (I) as well as dry storage (II) during 7½ months at 28°C. At 20°C 40% of the seeds germinated after wet storage and 38% after dry storage. The highest numbers of germinating seeds were obtained at 22.5°C and 25°C. These percentages were 84% and 80% respectively after wet storage and 69% and 70% respectively after dry storage. At 30°C the percentage was 72% after wet storage and 41% after dry storage. After wet storage of seeds of the 'greenhouse' strain (III) and the 'Westland' strain (IV) at 10°C during one month after shedding the results were similar, i.e. no germination at 15°C and 17.5°C, optimal germination at 25°C and a clear decrease in germination at 30°C. However, the percentages were relatively low, which could be attributed to the fact that the after-ripening was not yet completed. The percentages of germinating seeds of the 'Westland' strain were clearly lower than those of the 'greenhouse' strain.

The percentage of germinating seeds at 25°C after storage in water at various temperatures (28°, 10° and 4°C respectively) and different periods of time after shedding is shown in *fig. 2*. Directly after shedding the number of germinating seeds was very low, i.e. an average of 3% in both the 'greenhouse' strain and the 'Westland' strain. These numbers increased gradually in proportion to the time of storage and after circa 6 weeks a maxium was reached. The temperature during storage did not greatly affect the germination percentages with the exception of the 'Westland' strain seeds at 4°C where the germination percentage was relatively low after a period of eight weeks.

Table 1. Percentage of germinating *Pistia stratiotes* seeds (G) (at 15°C when submerged at various depths under different light intensities (the percentages refer to 100 tested seeds which had been shed more than eight weeks before treatment and stored in water of 10°C).

water depth (cm)	Light intensity ($erg.cm^{-2}.sec^{-1}$)	G (%)
25	0	0
25	900	9
25	4000	78
50	900	1
50	2000	4
75	900	0
75	1100	0
100	0	0
100	900	0

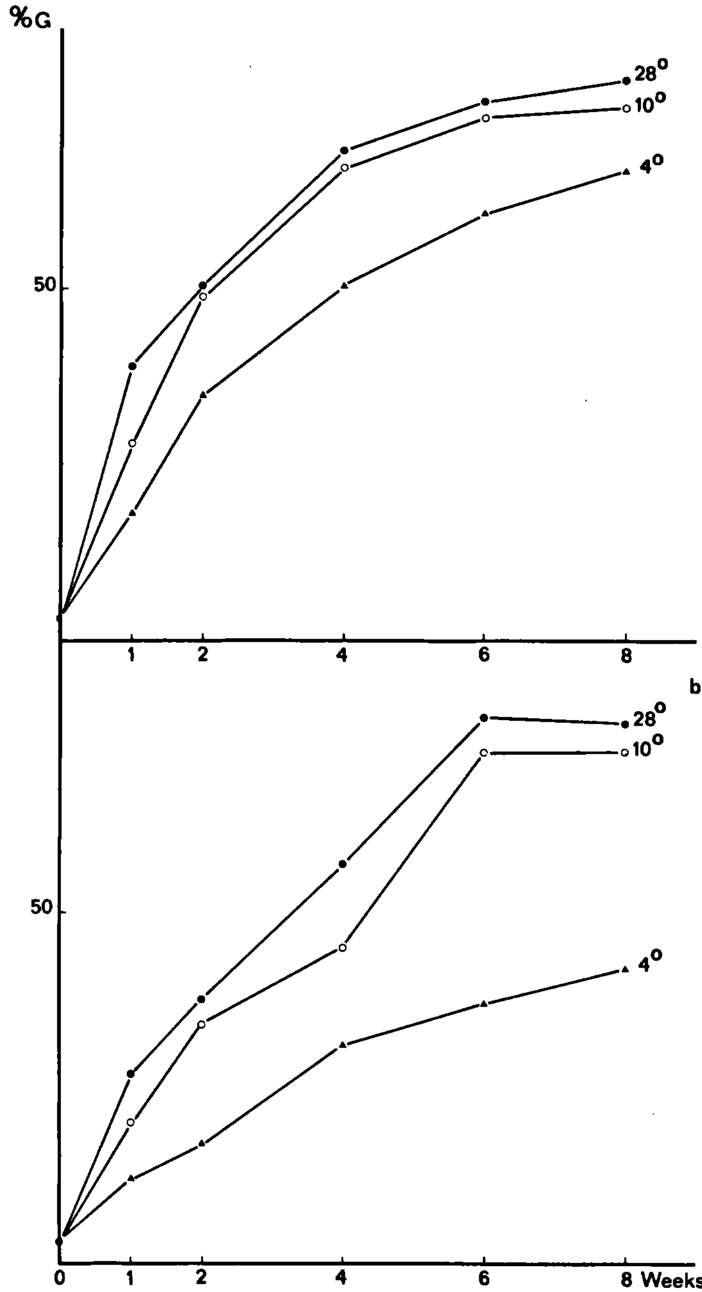

Fig. 2. Percentage of germinating *Pistia stratiotes* seeds (G) which directly after shedding were stored during different periods of time at a temperature of resp. 28 °C, 10 °C and 4 °C (the percentages refer to 100 seeds tested at 25 °C). a = 'greenhouse' strain; b = Westland strain.

The results of the experiment on seed germination (at 25 °C) after freezing the seeds in ice at –5 °C are shown in *fig. 3*. The percentage after half a week was the same as in the control (74%) but gradually decreased after longer storage in ice. After a period of four weeks the percentage germinating seeds was only 28% while there was no germination after 8 weeks in ice at –5 °C.

Pistia seeds were able to germinate if they were submerged, but the light intensity appeared to play an important role. The results are presented in *table 1*. At a depth of 25 cm there was a germination of 78% under a light intensity of 4000 erg.cm^{-2}.sec^{-1} but only 9% at a light intensity of 900 erg.cm^{-2}.sec^{-1}. At a depth of 50 cm under a light intensity of 2000 erg.cm^{-2}.sec^{-1} the germination was 4% and under a light intensity of 900 erg.cm^{-2}.sec^{-1} the germination was 1%. At a depth of 75 cm (light intensity respectively 1100 erg.cm^{-2}.sec^{-1} and 900 erg.cm^{-2}.sec^{-1}) and 100 cm (light intensity 900 erg.cm^{-2}.sec^{-1}) there was

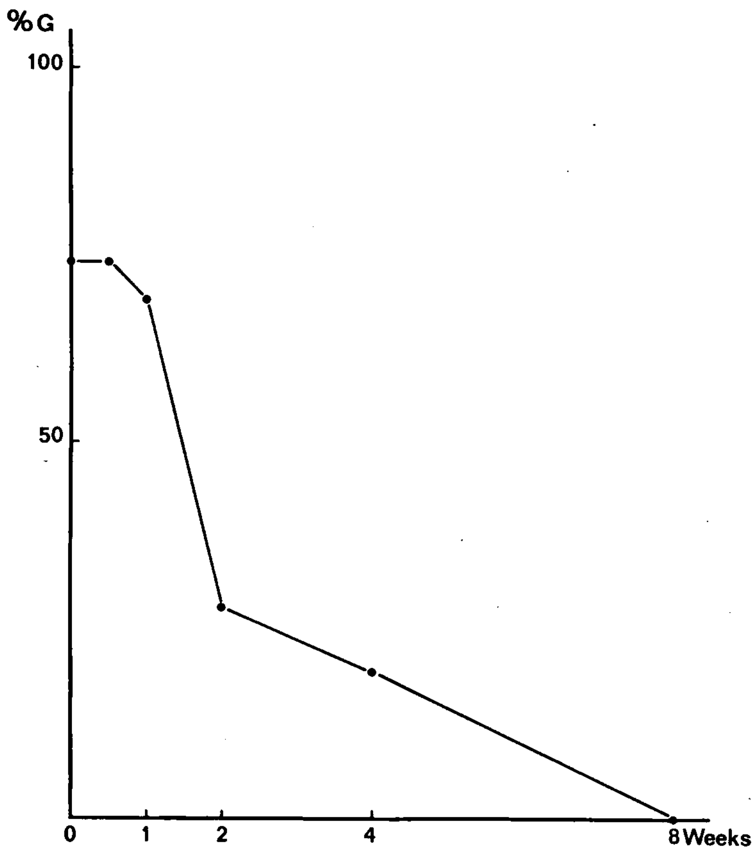

Fig. 3. The effect of freezing in ice at –5 °C during various periods of time on seed germination (G) of *Pistia stratiotes* (the percentages refer to 100 tested seeds which had been shed more than eight weeks before treatment and stored in water of 10 °C).

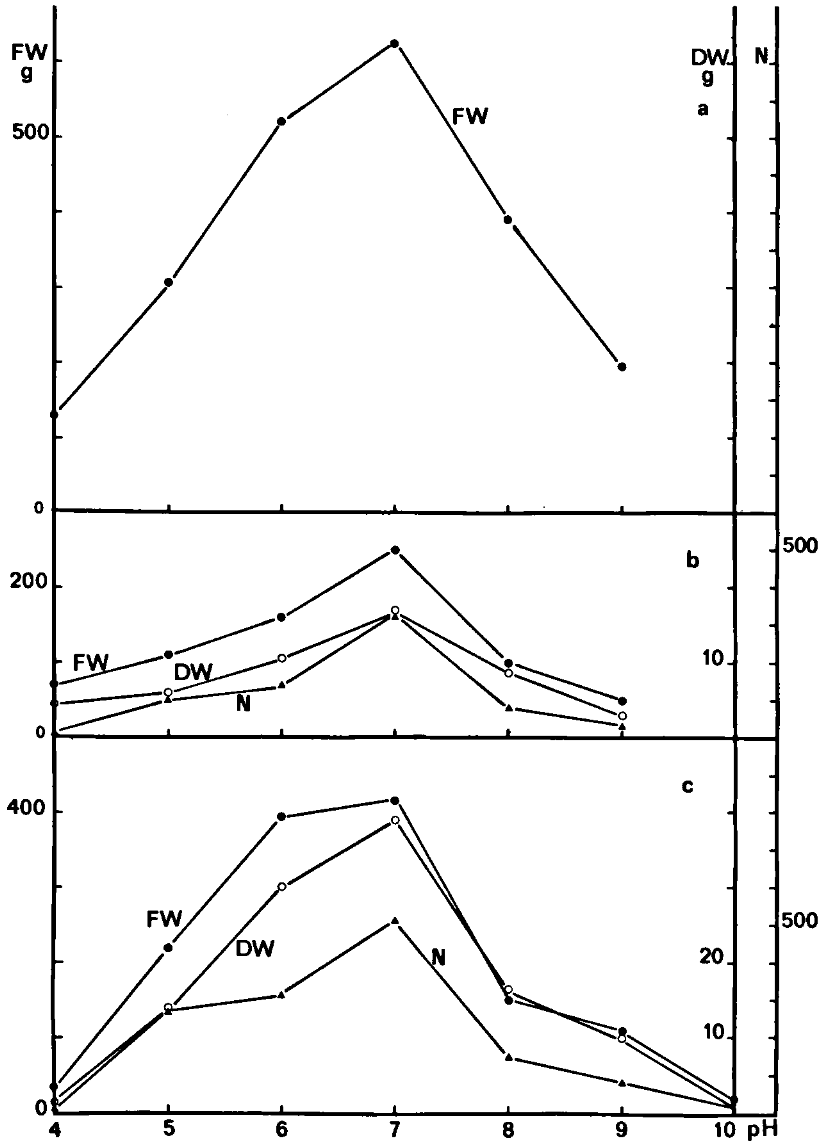

Fig. 4. The effect of pH on growth of *Pistia stratiotes* when cultivated on a) 1/5 strength Long-Ashton medium in an environmental chamber, b) tap water supplemented with micro and macro nutrients of 1/5 strength Long-Ashton medium in a greenhouse, c) half tap water/half rain water supplemented with micro and macro nutrients of 1/5 strength Long-Ashton medium in a greenhouse. FW = fresh weight; DW = dry weight; N = number of plants.

no germination. Germination was not observed either when the seeds were kept in the dark.

The results of the experiment on the effect of pH on *Pistia* are presented in *fig. 4*. The growth optimum was at pH 7 on 1/5 strength Long-Ashton medium in an environmental chamber as well as on combinations of 1/5 strength Long-Ashton medium with rain and half rain/half tap water in the greenhouse. Fresh weight, dry weight and number of plants decreased at higher and lower pH values (in the experiment in the environmental chamber only fresh weight was determined). However, under slightly acid conditions (pH 5–6) growth was better than under slightly alcaline conditions (pH 8–9). At pH 4 growth was strongly inhibited.

4. DISCUSSION

The results show that *Pistia* seeds are able to survive a prolonged period in water at 4°C and can even stand a few weeks in ice at –5°C. In general the water temperature at the bottom of ditches and canals in The Netherlands does not drop below 4°C and as a consequence it may be assumed that *Pistia* seeds will be able to survive the Dutch winter season. The germination of the seeds is restricted by the fact that light as well as a temperature of circa 20°C appear to be indispensable factors. During the spring the temperature at the bottom of Dutch water bodies will not reach this value and consequently the seeds will not germinate. However, in the middle of the summer, especially in shallow ditches a temperature of 20°C or higher is not uncommon. In this regard it may be assumed that germination of *Pistia* seeds, which were shed during the previous year, is possible in the following summer season in certain habitats. However, if the seeds become covered by mud or debris they will not germinate due to a lack of light. In this context it seems disadvantageous that in the middle of the summer the bottom of shallow water bodies will usually be covered with vegetation intercepting the light. When the seeds have germinated light is also necessary for the early development of the seedling, before it is able to rise up to the surface of the water. Summarizing it may be concluded that if seeds are produced during the summer season there is only a remote chance that plantlets will be produced during the following summer.

The question arises, however, whether under Dutch circumstances sufficient seeds could be produced in order to make it plausible from a statistical point of view that the species could bypass the ecological restrictions. In the greenhouse in Amsterdam the plants flower during the whole year, but in the field flowering specimens have only been observed during the hot summer of 1976. These plants were collected at a few sites in the 'Westland', where the vegetation was excessively dense. As a consequence it may be assumed that a relatively high temperature plays an important role in flower induction. In the laboratory the 'greenhouse' strain flowered under conditions of continuous light as well as under short days (8L : 16D) in the presence of certain chemicals, i.e. the chelating agent EDDHA (ethylenediamine-di-o-hydroxphenylacetic acid), salicylic acid or gibberellic acid (PIETERSE 1978). In this respect it might be hypothesized that water composition could also influence flowering. However, the rarity of seed formation under Dutch circumstances and the special conditions which are required for germination make it very unlikely that *Pistia* settles down in The Netherlands. On the other hand cooling water discharges and large scale dumping of *Pistia* plants from aquaria will certainly increase the possibilities for the weed to become integrated in the flora of Dutch waterways.

In general the percentage of germinating seeds of the 'Westland' strain was about the same as that of the 'greenhouse' strain or lower. There are no indications in connection with the present study to assume that the 'Westland' strain would be especially adapted to the Dutch climate.

DATTA & BISWAS (1970) reported that *Pistia* seeds do not germinate when they are submerged in 100 ml beakers which contain a layer of mud and are filled up to the border with tap water. However, the seeds started to germinate after the water had slowly evaporated and they concluded that submerged *Pistia* seeds did not germinate either because of a low oxygen or high CO_2 concentration. The observations of DATTA & BISWAS (1970) are not confirmed by the present study as submerged *Pistia* seeds germinated at a water depth of 25 and 50 cm, if they were exposed to a relatively high light intensity. DATTA & BISWAS did not mention the light intensity in their experiments, but some of the seeds were inbedded in mud and obviously were not exposed to light at all. In this context definite conclusions on the differences between the results of the study of DATTA & BISWAS and the present investigation are difficult to draw. However, as the water depth also appeared to exert an influence a low oxigen concentration might be a limiting factor to germination.

It appears that there is an after-ripening period of circa six weeks after the seeds are shed. However, this period did not appear to be an absolute requirement as directly after shedding a few seeds were already able to germinate and this amount increased gradually until after circa six weeks the maximum is reached. It should be noted that a 100% germination has never been obtained in the experiments and seed viability tests have not been conducted. The temperature during the period between the shedding of the seeds and the germination test did not appear to influence the length of the after-ripening period or the number of germinating seeds to a great extent. However, it is possible that in the 'Westland' strain the after-ripening period is longer than six weeks if the seeds are kept at 4°C as even after eight weeks the percentage of germinating seeds was lower than when the seeds had been stored at higher temperatures. Since the seeds had not been collected simultaneously various differences in seed viability could be expected. This might explain the discrepancies which appear in *fig. 1* and *fig. 2a* with regard to germination at 25°C after wet storage during 4 weeks at 10°C.

At 20°C the percentage germination was about the same for seeds stored under wet and dry conditions. However, at higher temperatures the percentages of germinating seeds was generally lower for seeds stored under dry conditions. This was most apparent at 30°C. It seems that after dry storage a number of the seeds have lost their viability and that more seeds are unable to germinate at a high temperature (30°C). However, in spite of this slightly negative effect of dryness on seed germination it may be assumed that in general *Pistia* seeds will be able to survive a prolonged period of drought.

On 1/5 strength Long-Ashton medium as well as on mixtures of tap water and half tap/half rain water with Long-Ashton medium there was a growth optimum of *Pistia* at pH 7, whereas at pH 4 growth was strongly inhibited. Consequently, these results contradict the observations of CHADWICK & OBEID (1966) who reported that *Pistia* had a growth optimum at pH 4 on 1/5 strength Long-Ashton medium as well as on tap water. In addition it seems questionable whether the pH is an important cause for water hyacinth crowding out *Pistia* in most natural water bodies, as was hypothesized by CHADWICK & OBEID (1966). This is further substantiated by a recent study by TAG EL SEED (1978), which showed that in mixed cultures with *Eichhornia crassipes* the competitive ability of *Pistia* at pH 4 was inferior to that at higher pH values.

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