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## Inhibition of seeds germination and radicle growth by glucose in silver maple (*Acer saccharinum* L.) seeds

### Abstract

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In the experiments fresh fallen silver maple seeds were used. Germination and growth of radicles can be inhibited by an extract from the seed coats at a concentration of 1 ml extract from one seed per one seed. Seeds coats in fresh state contain about 50 percent of sugars. Glucose at a concentration as in seed coats inhibits germination and growth of radicles. Mechanism of this inhibition is not known yet.

*Additional key words:* seed coats, sugars.

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### INTRODUCTION

Germination of seeds is one of the most studied but still not completely understood physiological phenomenon. A very large number of substances can inhibit germination. Most interesting is the action of substances which prevent germination without affecting the seeds irreversibly. The most important germination inhibitor known is undoubtedly abscisic acid. The presence of abscisic acid has been reported in seeds of sugar maple (Rudnicki and Suszka 1969) but ABA is clearly not the only natural growth inhibitor (Tomaszewska 1979).

The purpose of this research was to continue the studies of Tomaszewska (1979) from the point of view of a closer examination of the activity germination inhibitors in non-dormant silver maple seeds. The content of sugars and influence of glucose on the germination of silver maple seeds were studied.

### MATERIAL AND METHODS

#### PLANT MATERIAL

Seeds of silver maple were collected in Kórnik Arboretum after fruit dispersal in the first days of June. Seeds destined for the experiments were removed from samaras. In the experiment, intact seeds or naked seeds were germinated on

a glucose solution at concentration up to 0.5 M or on an extract from the seed coats. The radicle length was measured at 24 hour intervals.

**Water content.** Fresh and dry weight of seeds, seeds coats and radicles was recorded after 24 h of drying at 105°C and the water content of the seeds was calculated in relation to fresh weight.

**Electrolytes leakage.** For the leaching test fresh collected seeds were used. Three samples of seeds for each glucose concentration were dipped in 10 ml of redistilled water and held at room temperatures. Electrical conductivity was measured every 6 hours (during 48 h) using a conductometer OK-102/1. Then, after boiling of seeds in a new 10 ml of water the total content of electrolytes was determined. Before extraction, seeds were surface-washed by water. The percent of leaching electrolites was calculated. This measurement was repeated three times and presented as percent of electrolytes leakage.

**Protein determination.** Protein content in leachate from seeds was determined by the Lowry method (Lowry et al. 1951) and calculated as percent of protein in dry weight of seeds.

**Sugars content determination.** Total sugars in the seeds was determined by the phenol method (Montgomery 1961) and calculated as percent of fresh weight per one organs. Reducing sugars were determined by the Somogyi (1952) and Nelson (1957) methods. Three replicates for each determination were used.

All data were analysed statistically.

## RESULTS AND DISCUSSION

### GERMINATION OF SILVER MAPLE SEEDS

Silver maple is one of the few species in the genus *Acer* which yield non-dormant seeds at dispersal time, capable of germination immediately after ripe seeds fall off the tree.

As we see from Fig. 1, embryo (cotyledons and radicle) makes up the greatest part of the seed—above 90 percent of fresh weight of seed. During imbibition, water is taken up by the cotyledons, but not by the seed coat. After 4 days, when epicotyl starts to grow, fresh weight of seed coat slowly decreases, but after 7 days weight of seed coat was minimal. The seeds on water start to germinate after 2 days (Fig. 2) and after the next 2 days all sound seeds are germinated. Radicles start growth after 2 days. After 6 days, the fresh weight was about 10 times higher (Fig. 1).

### INFLUENCE OF WATER EXTRACT FROM SEED COATS ON GERMINATION

Silver maple seeds without seed coats germinate similarly as with them (Fig. 2). No stimulation or inhibition was observed. Growth of radicle was faster when the seed coats were removed (Fig. 3). When seed coats were extracted by hot

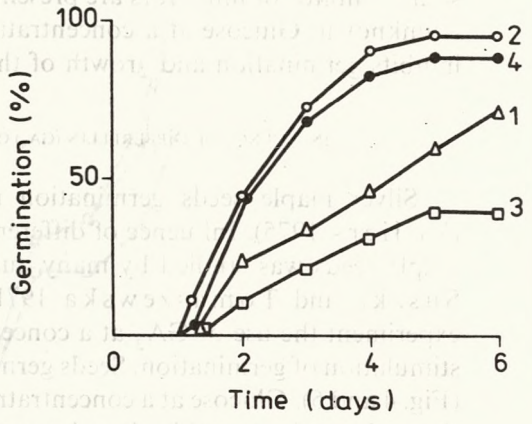
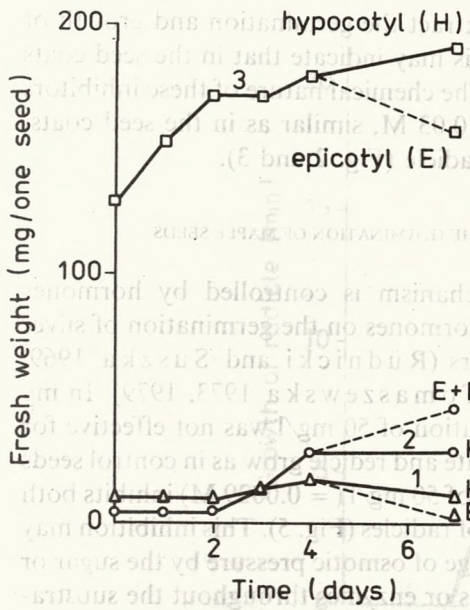


Fig. 1. The change of mean fresh weight of seed coat (1), radicle (2) and cotyledons (3) during germination of *A. saccharinum* seeds

Fig. 2. Germination of *A. saccharinum* seeds on glucose solution (3), water extract from seeds coats (1), water (2) and seeds without coats on water (4)

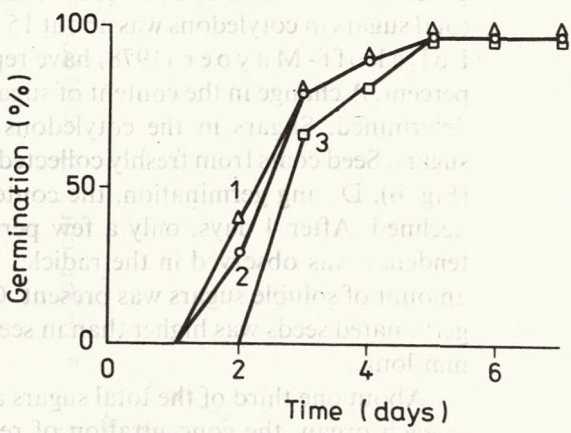
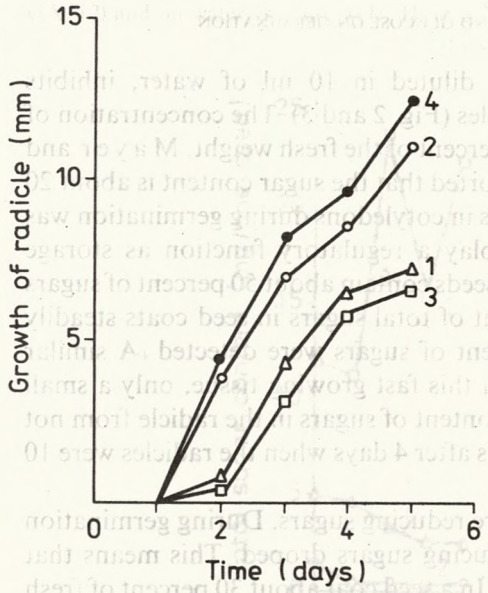


Fig. 3. Growth of radicle during germination of *A. saccharinum* seeds on glucose (3), on water extract from seed coats (1), on water (2) on water without seeds coat (4)

Fig. 4. Germination of *A. saccharinum* seeds on glucose (50 mg/l) (3), on  $GA_3$  (50 mg/l) (2) and on water (1). These difference was shown to be statistically significant in Student's test

water and seeds were put on the water extract the germination and growth of radicles were inhibited (Fig. 2 and 3). This may indicate that in the seed coats some inhibitor or inhibitors are present. The chemical nature of these inhibitors is unknown. Glucose at a concentration 0.03 M, similar as in the seed coats, inhibits germination and growth of the radicle (Fig. 2 and 3).

#### INFLUENCE OF GIBBERELLIN (GA<sub>3</sub>) ON THE GERMINATION OF MAPLE SEEDS

Silver maple seeds germination mechanism is controlled by hormones (Villiers 1975). Influence of different hormones on the germination of silver maple seeds was studied by many authors (Rudnicki and Suszka 1969, Suszka and Tomaszewska 1971, Tomaszewska 1973, 1979). In my experiment the use of GA<sub>3</sub> at a concentration of 50 mg/l was not effective for stimulation of germination. Seeds germinate and radicle grow as in control seeds (Fig. 4 and 5). Glucose at a concentration of 50 mg/l (= 0.0029 M) inhibits both the seed germination (Fig. 4) and growth of radicles (Fig. 5). This inhibition may be explained as a consequence of the change of osmotic pressure by the sugar or by the influence of the sugar on hormones or enzymes throughout the substrate-product feedback mechanism. When glucose was applied to seeds at a concentration as in the extract, it inhibited growth of roots similarly as the extract alone (Fig. 5 and 6).

#### INFLUENCE OF SUGAR CONTENT AND GLUCOSE ON GERMINATION

Water extract from 10 seed coats, diluted in 10 ml of water, inhibits germination of seeds and growth of radicles (Fig. 2 and 3). The concentration of total sugars in cotyledons was about 15 percent of the fresh weight. Mayer and Poljakoff-Mayer (1978) have reported that the sugar content is about 20 percent. A change in the content of sugars in cotyledons during germination was determined. Sugars in the cotyledons play a regulatory function as storage sugars. Seed coats from freshly collected seeds contain about 50 percent of sugars (Fig. 6). During germination, the content of total sugars in seed coats steadily declined. After 4 days, only a few percent of sugars were detected. A similar tendency was observed in the radicle. In this fast growing tissue, only a small amount of soluble sugars was present. Content of sugars in the radicle from not germinated seeds was higher than in seeds after 4 days when the radicles were 10 mm long.

About one third of the total sugars are reducing sugars. During germination in each organ, the concentration of reducing sugars dropped. This means that reducing sugars are rapidly metabolized. In a seed coat about 30 percent of fresh weight there are reducing sugars. During germination the content of sugars rapidly drops.

In radicles, the higher concentration of sugars was detected after 24 hours of germination. When the radicles were about 10 mm after 4 days, only a small

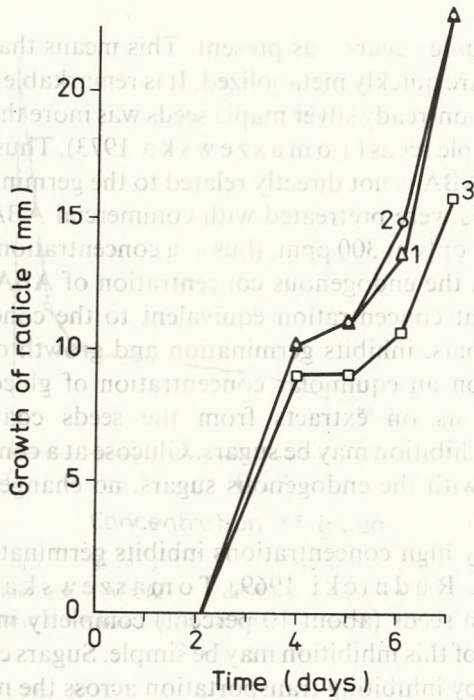


Fig. 5. Growth of radicle during germination of *A. saccharinum* seeds on glucose solution (3), on  $GA_3$  (2) and on water as control (1). These difference was shown to be statistically significant in Student's test

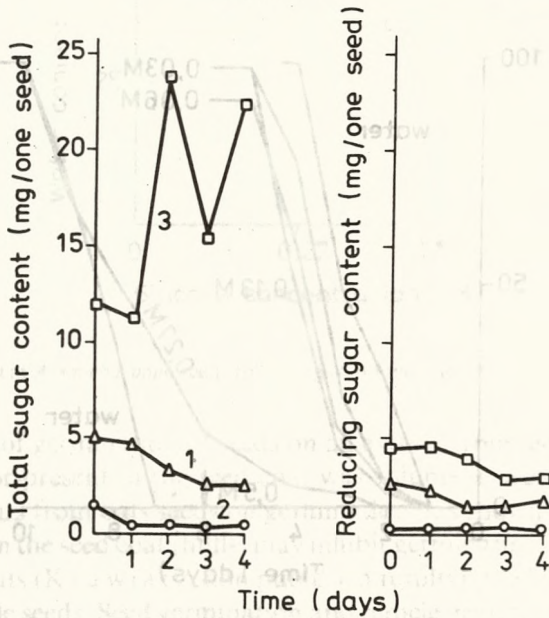


Fig. 6. Change of the content of total and reducing sugars in cotyledons (3), seed coats (1) and in radicles (2), during germination of *A. saccharinum* seeds

concentration of soluble sugars was present. This means that soluble sugars in fast growing organs are quickly metabolized. It is remarkable that the amount of ABA in the germination-ready silver maple seeds was more than twice that in the dormant Norway maple seeds (Tomaszewska 1973). Thus it appears that the absolute amount of ABA is not directly related to the germination status. When the silver maple seeds were pretreated with commercial ABA, the germination was severely restricted only at 300 ppm, thus at a concentration of ABA exceeding more than 200 times the endogenous concentration of ABA at dispersal time. Exogenous glucose at concentration equivalent to the concentration of total sugars in the seed coats, inhibits germination and growth of radicles of silver maple seeds. Also, on an equimolar concentration of glucose solution, seeds germinate similarly as on extracts from the seeds coat. The substances responsible for this inhibition may be sugars. Glucose at a concentration (0.5 M), which is equimolar with the endogenous sugars, no changes water content 48 hours after application.

ABA only at very high concentrations inhibits germination of silver maple seeds (Suszka and Rudnicki 1969, Tomaszewska 1979). Glucose at a concentration as in seeds (about 10 percent) completely inhibits germination (Fig. 7). Mechanism of this inhibition may be simple. Sugars can act as regulators of osmotic pressure by inhibiting transportation across the membrane. Glucose alone completely stops germination. After removing glucose by 30 minutes, washing, seeds germinate rapidly as do control seeds (Fig. 7). Glucose retards germination without injury of seeds.

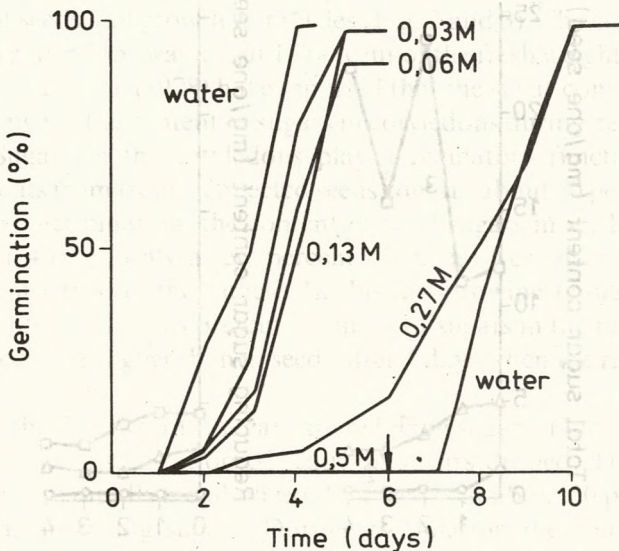


Fig. 7. Germination of *A. saccharinum* seeds on a glucose solution at different concentrations. Glucose at a concentration of 0.5 M completely inhibited germination. Arrow indicates a 30 minutes washing out glucose by water.

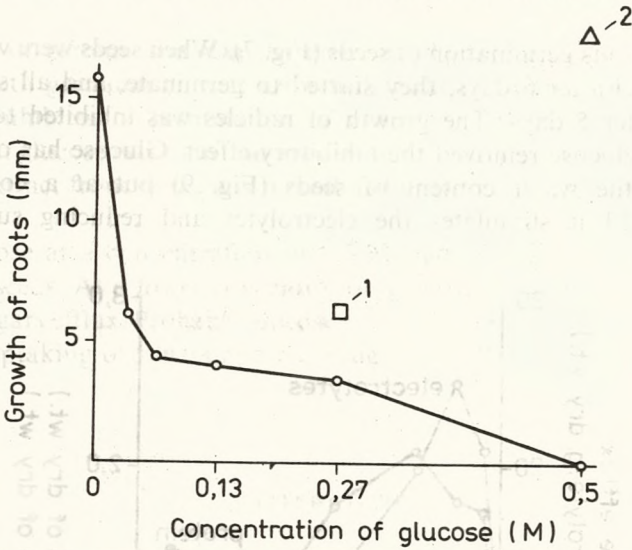


Fig. 8. Growth of radicles of *A. saccharinum* seeds of different glucose solutions (1) — after 11 days on glucose (0.2 M), (2) — 6 days on glucose followed by washing with water and (0.5 M) 5 days germination on water. These difference was shown to be statistically significant in Student's test

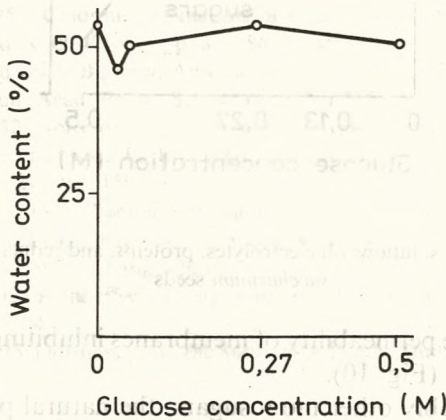


Fig. 9. Water content in *A. saccharinum* seeds following 48 hours of germination on glucose solutions

Observation of germination of seeds on an extract from seed coats indicates that the inhibitor present in the seed coat was soluble in water. A study of the influence of sugars from oats seeds on germination has shown that maltose and glucose present in the seed coat (hulls) may inhibit germination in concentrations as in the seed coats (Krawierz, non published results). We have a very similar situation in maple seeds. Seed germination and radicle growth on a water extract showed that glucose at a concentration as in seed coat inhibited these processes (Fig. 7 and 8). Glucose at a concentration of 0.5 M which is an isotonic solution

completely inhibits germination of seeds (Fig. 7). When seeds were washed with water (30 min.) after 6 days, they started to germinate, and all sound seeds germinated after 5 days. The growth of radicles was inhibited too (Fig. 8). Removing of glucose removed the inhibitory effect. Glucose has only a small influence on the water content of seeds (Fig. 9) but at a concentration of 0.05—0.1 M it stimulates the electrolytes and reducing sugars efflux (Fig. 10).

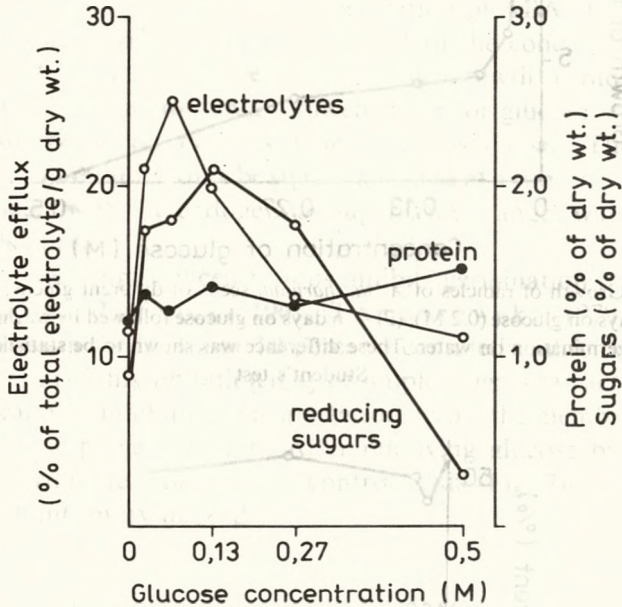


Fig. 10. Influence of glucose solutions on electrolytes, proteins, and reducing sugars efflux from *A. saccharinum* seeds

Glucose acts on the permeability of membranes inhibiting sugars uptake and reducing sugars efflux (Fig. 10).

Glucose and probably other monosugars, the natural products of metabolism, play an important role in the regulation of germination and growth of seedlings.

#### CONCLUSION

1. Silver maple seeds germinate immediately after ripe seeds fall of the tree. Water extract from the seed coats inhibits germination and growth of radicles. Chemical nature of this inhibitor (-s) is unknown. Seed coats from freshly collected seeds contain about 50 percent of sugars.

2. The use of  $GA_3^*$  at a concentration of 50 mg/l was not effective in

\* List of abbreviations: ABA — abscisic acid,  $GA_3$  — gibberellic acid.



stimulation of germination. On the other hand glucose at this concentration inhibited both germination and growth of radicles. ABA is known to be an ineffective inhibitor of germination of silver maple seeds (Tomaszewska 1979). When glucose was used and applied to seeds at a concentration similar to that as in extract from seed coats it inhibited growth of roots just as the extract did.

3. Glucose at a concentration of 0.5 M had no influence on total water content of seeds. At a lower concentration glucose stimulates electrolytes and reducing sugars efflux. Probably glucose acts on the permeability of membranes, inhibiting uptaking of sugars and reducing sugar efflux.

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#### Wpływ glukozy na kiełkowanie i wzrost korzenia zarodkowego nasion klonu srebrzystego (*Acer saccharinum* L.)

##### Streszczenie

Wodny wyciąg z okryw nasiennych przylegających do zarodka w stężeniu 1 ml wyciągu na 1 nasiono powoduje hamowanie kiełkowania i wzrost kielków zarodkowych nasion klonu srebrzystego *Acer saccharinum* L.

Okolo połowy suchej masy tych okryw stanowią związki cukrowe. Glukoza w takim stężeniu, w jakim występuje w okrywkach, hamuje kiełkowanie nasion i wzrost kielków zarodkowych. Mechanizm tego hamowania nie jest wyjaśniony.

**Влияние глюкозы на прорастание и рост зародышевого корешка семян клена сахаристого (*Acer saccharinum* L.)\***

**Резюме**

Водная вытяжка из семенных оболочек, прилегающих к зародышу, в концентрации 1 мл вытяжки на 1 семя приводит к торможению прорастания и росту зародышевых ростков семян клёна сахаристого (*Acer saccharinum* L.).

Около половины сухой массы этих оболочек составляют сахаристые соединения. Глюкоза в такой концентрации в какой находится в оболочках, тормозит прорастание семян и рост зародышевых ростков. Механизм этого торможения не выяснен.

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