ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XXXVII — nr 1
1968

Inhibitory effect of apple juice on the germination of apple and cherry seeds and the growth of apple seadlings

W. KAMIŃSKI

Bartlett (1961) and Come (1962, 1965) found that in the seed coats of apple seed obtained from fruits stored for 2—3 months occured probably chlorogenic acid (Come 1965), which inhibited seed germination in apples. Apple flesh was a source of the inhibitor. The authors mentioned above, did not observe any harmful influence of the inhibitors on the germination of apple seed if its seed coats removed. On the other hand, many nurserymen and plant breeders Kopan (1967) noticed a poor growth of seedlings obtained from the seeds taken from fruits stored for several months.

In this experiment, trials were made to establish the influence of the inhibitors present in apple flesh on the germination of seeds without seed coats, and on the subsequent growth of seedlings. An effort was also made toward identification of the inhibitor present in the apple flesh.

MATERIAL AND METHODS

The seeds from the apple variety 'Idared' stored at 4°C for 6.5 months were used. Apple juice was pressed out from the same fruits. The seed coats and endosperms were removed from part of the seeds, and the embryos were transferred then to Petri dishes and placed on filter paper soaked in water. This treatment was considered as a check (1). The rest of these seeds was placed in the filter paper previously soaked in apple juice. About 10 mililiters of apple juice were added to each Petri dish of 10 centimeters in diameter. Except for the check treatment the following combinations were set up:

Seeds without integuments (2);

Seeds without integuments and endosperm (3);

Whole seeds (4).

The treated seeds were kept at a temperature of about 20°C. After five days of storage the seeds from teatment (3) were deprived of endosperms and the seeds from treatment (4) had their seed coats and endosperms removed. The embryos from treatments (2), (3) and (4) were transferred then to Petri dishes lined with filter paper soaked in water. On the 10th

day of the experiment, all embryos were planted in soil and left in the greenhouse at 25°C. The experiment was repeated 3 times using 3 Petri dishes with 20 seeds in each dish. The results were analysed statistically with the method of analysis of variance using the "t" test for significance.

Apple juice was extracted with ether according to the method of Cornforth et al (1965). After evaporation the residue was dissolved in potassium hydrocide, treated with lead acetate and line loaded on Whatman No 3 paper. A chromatogram was developed in isopropanol--ammonia-water (10:1:1/v/v/v). The presence of the inhibitor on the chromatografic paper was established by straight growth wheat coleoptiles section test.

RESULTS

Apple juice strongly inhibited seed germination. After 5 days the number of germinated seeds was 40 per cent lower than the seeds treated with water (Table 1) Similarly, the number of seedlings obtained from the seeds treated with apple juice was much lower than from the control seeds.

Table 1

The effect of apple juice on the germination of apple seeds and the growth of apple seedlings

Treatment Kombinacja		Per cent of germinated seeds after 5 days. Procent skiełkowanych nasion po 5 dniach	Per cent of seedlings obtained Procent uzyskanych siewek	Average height of seedlings (in cm) Przeciętna wysokość siewek (w cm)	
				After 2 months Po 2 miesiącach	After 4 months Po 4 miesiącach
	Check (1) Kontrola (1)	90.0	96.6	10.4	11.8
Soaked in apple juice Moczone w soku jabłkowym	Embryos without endosperm (2) Zarodki bez bielma (2)	46.6	75.0	5.0	9.7
	Embryos with endosperm (3) Zarodki z bielmem (3)	45.0	86.6	5.7	9.2
	Whole seeds (4) Całe nasiona (4)	33.0	71.0	4.7	6.6

The seedlings obtained from all seeds proviously soaked in apple juice showed weak growth, (Tab. 1) and had short internodes but they did not differ significantly after two months of growth. The difference between seedlings obtained from seeds treated with apple juice and from check seeds was very significant. This difference got smaller (with the exception of treatment 4), but is was still significant after 4 months of growth.

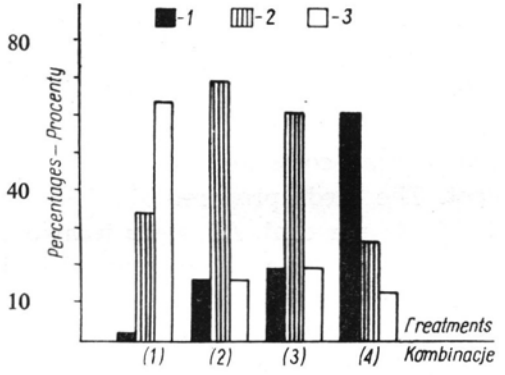

Fig. 1. Characteristics of apple seedlings after 4 months of their growth.
Charakterystyka siewek jabłoni po 4-ch miesiącach ich wzrostu

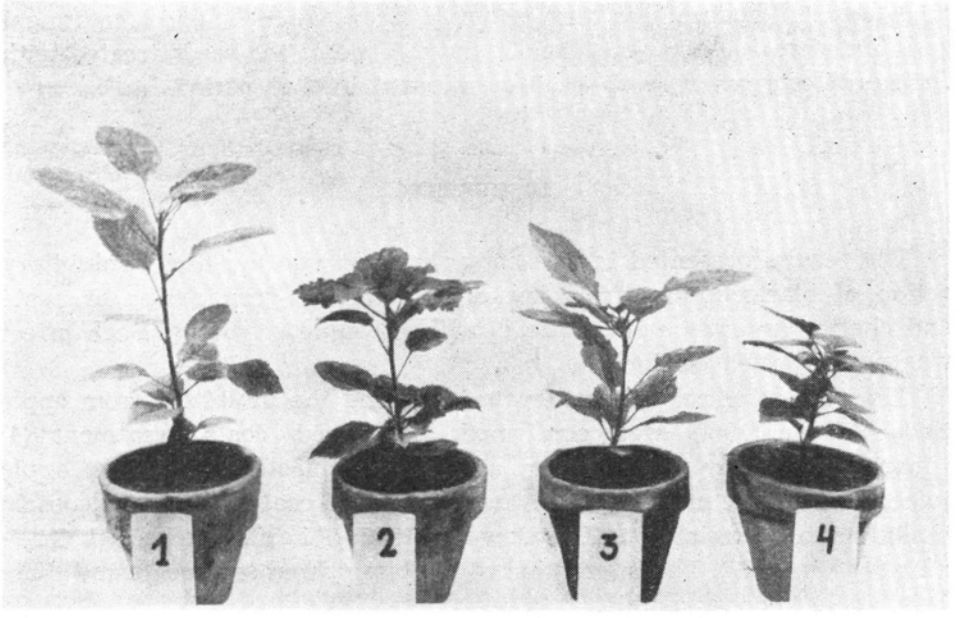

Fig. 2. General appearance of control seedling (1) and those obtained from seeds treated with apple juice (treatments 2, 3 and 4), after 4 months of growth.

Ogólny wygląd siewek kombinacji kontrolnej (1) oraz siewek kombinacji traktowanych sokiem (2), (3), (4) po 4 miesiącach wzrostu

Number and size of leaves of the seedlings from all four treatments were very similar.

The difference in growth of seedlings obtained from differently treated seeds is shown in Fig. 1 and Fig. 2. The measurements of the seedlings were made after four month of growth. A very small per cent of seedlings obtained from the check treatment showed rosette type of growth. Marked inhibition of growth occurred in the group of seedlings from the treatment (4). Plants obtained from treatments (2) and (3) had an intermediate type of growth.

Preliminary experiments indicated also the inhibitory action of apple juice on the germination of sweet cherry seeds (*Prunus avium*). Stratified cherry seeds, deprived of seed coats and endosperms, germinated after 10 days in 90 per cent. The seeds prepared similarly but treated with apple juice germinated in 46 per cent. All seeds treated with apple juice had much shorter hypocotyles than those from the check treatment. The germinated seeds were planted in pots and grown in the greenhouse. However, the difference in height between juice treated and untreated seedlings dissappeared after two weeks.

Experiments with chromatographically purified apple juice have revealed an inhibitor af R_f 0.65—0.80 which suppressed the growth of wheat coleoptiles (about 80 per cent growth inhibition). The isolated substance inhibited also the germination of apple seeds from which the seed coats and endosperms were removed. Seeds from the check treatment germinated in 100 per cent after 10 days. None of the seeds treated with the inhibitor from R_f 0.65—0.80 germinated in that period.

DISCUSSION

The results presented in this paper have proven the direct inhibitory action of blastocholines from apple juice on the germination of apple and cherry embryos and on the growth of apple seedlings. Such effect was not observed earlier.

The particular role of seed coats in storing the inhibitors from apple juice Côme (1962) was confirmed. The seed coats treatment (4) enhanced markedly the inhibitory action of the blastocholines from apple juice. It should be mentioned, however, that seed coats themselves contain inhibitors of germination (Luckwill 1952; Pieniążek, and Grochowska 1967). The interaction of inhibitors from seed coats and those from apple juice is possible.

Apple seedlings obtained from seeds treated with juice were of similar appearance to seedlings obtained from seeds which did not undergo the afterripening. It is then probable that the inhibitor from apple juice plays a role in the process responsible for the dormancy of the seeds.

These results throw a light on the causes of weak growth of seedlings obtained from apples stored for a long time. It is interesting that the inhibitor from apple juice suppresses also germination of cherry seeds.

The inhibitor found in this experiment has a R_f value similar to the inhibitor found in peach seeds — Abscisin II (Lipe 1966). Abscisin II was found recently in apple juice (Cornforth, personal comunication to J. Pieniążek) and in leaves and apple juice (Pieniążek, Rudnicki 1967).

The obtained results suggest that the substance inhibiting germination of apple and cherry seeds and growth of apple seedlings is Abscisin II a hormone known to induce dormancy in woody plants (Thomas, Wareign, Robinson 1965).

I am intebted to Mr R. Rudnicki for his help in the isolation of the inhibitor.

SUMMARY

Apple seeds of the variety Idared stored for 6.5 months at 4°C and afterripened sweet cherry were treated with apple juice. The treatment reduced the germination of apple and cherry seeds and also the growth of apple seedlings. Apple seedlings obtained from seeds treated with juice showed rosette type of growth similar to those obtained from seeds which did not pass after-ripening. The most evident inhibition of growth occured in the group of seedlings which were obtained from whole seeds treated with apple juice.

Chromatography of apple juice revealed the existance of a substance which inhibited elongation of wheat coleoptile and germination of apple seeds. The R_f of the substance was very similar to the R_f of a hormane Abscisin II.

Department of Plant Breeding
Institute of Pomology
in Skierniewice

(Entered: July 15, 1967.)

Hamujący wpływ soku z jabłek na kiełkowanie nasion jabłoni i czereśni oraz na wzrost siewek jabłoni

Streszczenie

Nasiona jabłoni odmiany 'Idared' przechowywane w owocach w temp. 4°C przez 6,5 miesiąca, oraz stratyfikowane nasiona czereśni ptasiej poddawano działaniu soku z jabłek. W wyniku tego zabiegu otrzymano silne zahamowanie kiełkowania zarodków jabłoni i czereśni oraz zahamowanie późniejszego wzrotu siewek jabłoni. Uzyskane siewki jabłoni charakteryzowały się rozetkowym wzrostem podobnym do siewek uzyskiwanych z nasion, które nie przeszły procesu posprzętnego dojrzewania. Najbardziej zahamowane we wzroście były siewki jabłoni uzyskane z kombinacji, gdzie całe nasiona traktowano sokiem. Za pomocą chromatografii soku znaleziono czynnik powodujący bardzo silne zahamowanie wydłużania koleptile pszenicy i kiełkowania nasion jobłoni. R_f inhibitora było zbliżone do R_f hormonu Abscysyny II.

LITERATURE

Bartlet C. E., 1961, The after-ripening of apple seeds in the fruit during cold storage. The Ann. Rep. Agr. and Hort. Long. Asthon Bristol 66—67.

Côme D., 1962, Germinations des pepins, de pommes conserves a diverses temperatures, XVI International Horticultural Congress, Brussels—Belgium.

Côme D., 1965, Influence de la temperatures sur les taux et la vitesse de germination des graines de Pommier (*Pirus malus* L.) tegumentarre, C. A. R. Acad. Sc. Paris 360—1725—1728.

Cornforth I. W., Milborrow B. V., Ryback G., Waering P. F., 1965, Chemistry and physiology of "Dormins" in sycamore, Nature 205:1269—73.

Kopan W., 1967, Chranit li siemiena w płodach, Sadowodstwo 1—22.

Luckwill L. C., 1952, Growth inhibiting and growth promoting substances in relation to the dormancy and after-ripening of apple seeds, J. Hort. Sci. 27: 53—67.

Pieniążek J., Grochowska M., 1967, The role of the natural growth inhibitor (abscisin II) in apple seed germination and the changes in the content of phenolic substances during stratification. Acta Soc. Bot. Pol. 36, 3:579—587.

Pieniążek J., Rudnicki R., 1967. The presence of abscision II in apple leaves and fruit juice. Bull. Acad. Polon. Sci. 15,5:251—254.

Thomas T. H., Wareing P. E., Robinson P. M., 1965, Action of the sycomore. Dormin as a gibberelin antagonist. Nature 205—1270.