ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 50, nr 4: 663—672
1981

Generative reproduction of *Vaccinium myrtillus* in laboratory conditions and the influence of zinc-smelter emissions on it

ZUZANNA CZUCHAJOWSKA, TERESA STRĄCZEK

Department of Plant Ecology, Institute of Ecology of Highly Industrialized Regions, Silesian University, Jagiellońska 24, 40-032, Katowice, Poland

(Received: October 27, 1980)

Abstract

The germination of *Vaccinium myrtillus* seeds and the development of the seedlings in laboratory conditions are described. The dynamics, the power of germination and Pieper coefficients for the seeds were strongly influenced by the intensity of zinc-smelter pollution of the forest stand from which they originated. The rates of survival were also estimated for seedlings transferred from filter-paper into polluted and unpolluted samples of soil and litter. The differences noticed in the latter parameter point to the possibility of formation in the polluted stand of ecotypes more resistant to the influence of pollution than the original ones.

INTRODUCTION

There are only few incomplete data in the literature concerning generative reproduction of *Vaccinium* species and particularly of the ecology of *Vaccinium myrtillus* seedlings (Kozirackі and Targoński 1978). The authors undertook, therefore, laboratory investigations of the germination of this species and described the seedlings grown during three years. The experience gained in this way was afterwards utilized in research of the influence of zinc-smelter emissions on the generative reproduction of *Vaccinium myrtillus*. In particular the dynamics and the power of germination of the seeds and the survival rates of the seedlings depending on the pollution level of the stands were investigated.

MATERIAL

The seeds of *Vaccinium myrtillus*, the germination of which on filter-paper and on soil and litter samples was studied in the laboratory, originated from three stands of a forest ecosystem (Żyglinek, Brynica

II and Kokotek), representing different pollution degrees by the emissions of the neighbouring zinc-works at Miasteczko Śląskie, mainly by some heavy metals (Pb, Zn, Cd, Cu) contained in the dustfall (Czuchajowska et al. 1980, Czuchajowska 1981). The investigations were carried out in 1978-1980, in the same manner as described for *Vaccinium vitis-idaea* (Czuchajowska and Strączek 1981). The mixture of soil and litter samples, collected from the three investigated stands in which the seedlings primarily developed on filter-paper were transferred, contained both components in a 1 : 1 ratio by volume.

All laboratory germinations were carried out in four replications. The following determinations were made: 1) the percentage of seeds germinating on every subsequent day, called the germination dynamics, 2) germination power, 3) Pieper coefficients giving the average number of days required for single seed germination and 4) survival rates of seedlings, described by the percentage of the total number of seedlings which survived after a given number of days. The reproducibility of results was excellent, the data in Figs. 2-4 and Table 1 represent mean values.

RESULTS

Several years observation of seeds germinating on filter-paper in optimum conditions, show that an epigenic germination occurred. No differences between the seeds originating from the control area and the polluted ones were found. The radicle emerged from the seed around the 10th day, preceeding by about 14 days the appearance of the epicotyl outside the seed coat. The cotyledons were fully developed 20-25 days after sowing. The hypocotyl was then 1 cm in length, the cotyledons

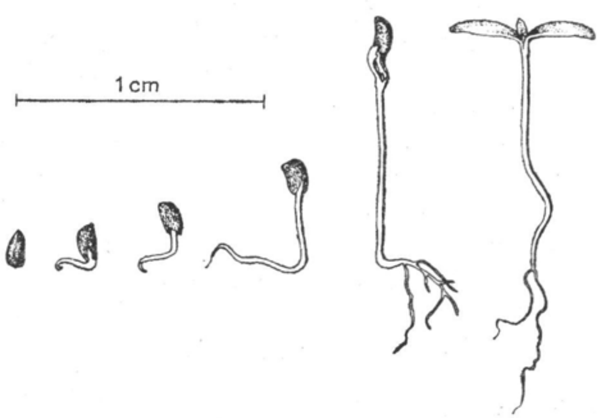

Fig. 1a Germination of *Vaccinium myrtillus* seeds and seedlings after 10, 15, 20, 25 and 30 days

were green with a smooth margin. They were on the average about 3 mm in length and 1 mm in width. The main root was 1 cm long, it possessed 5-10 ramifications on the average. Around the 40th day the first leaf appeared, which was ovate and serrate. The formation of every subsequent leaf took 7 to 10 days. After about 2 months a bud in the axil of one of the cotyledons appeared and started to elongate faster than the main shoot (Fig. 1a).

The seedlings grown in the laboratory did not shed leaves. This was at first attributed by the authors to the artificial conditions of cultivation. However, it was found later that the one- two- or even three--year-old seedlings of *Vaccinium myrtillus* of generative reproduction origin, also kept their leaves in natural conditions. Thus, it appeared that the seedlings behave differently than the vegetatively reproducing plants of this species. The one- and two-year-old seedlings growing in natural, pollution-free stands, had the same appearance as those grown in the laboratory on soil from the control area. They were only distinctly smaller (Fig. 1b). Also smaller, though not to the same extent, were seedlings grown in the laboratory on polluted soils. Among the seedlings from the natural stand, formation of rhizomes during the third year was observed, indicating a possibility of vegetative propagation.

The dynamics of germination on filter-paper show that the newly collected seeds representing a highly differentiated level of pollution with heavy metals (Czuchajowska 1981) are characterized by an identical time-pattern of germination and differ only slightly in the percentage of seeds which germinated on the given days (Fig. 2a). In every case the percentage of germinating seeds decreased below 2% in the 19th day. Storage of the seeds for 3 months at 2° C, changed the dynamics of germination. The seeds with a definite level of pollution showed now only one dominating maximum of germination, which was shifted by 2-3 days as compared to that shown previously. The period after which the germination ability disappears is prolonged by a few days (Fig. 2b). A 6-month storage of the seeds at 2° C results in a decrease of the intensity of maxima, e.g. for the control seeds by 50% on the 17th days and for the extremely polluted ones by 40%. An increase in the storage temperature from 2° C to 20° C does not influence the germination dynamics (Fig. 2c). When the germination of seeds on soil taken from the same stand was delayed by 3 months, it differed markedly from the process carried out on filter paper (Fig. 2d, cf. Fig. 2b).

Substitution of soil by litter from the same stand from which the seeds were taken, does not change essentially the time-pattern of germination. It only decreases the intensity of the early appearing maxima often by one half.

Fig. 1b. Two-year-old seedlings grown in laboratory (left) and in natural stand (right)

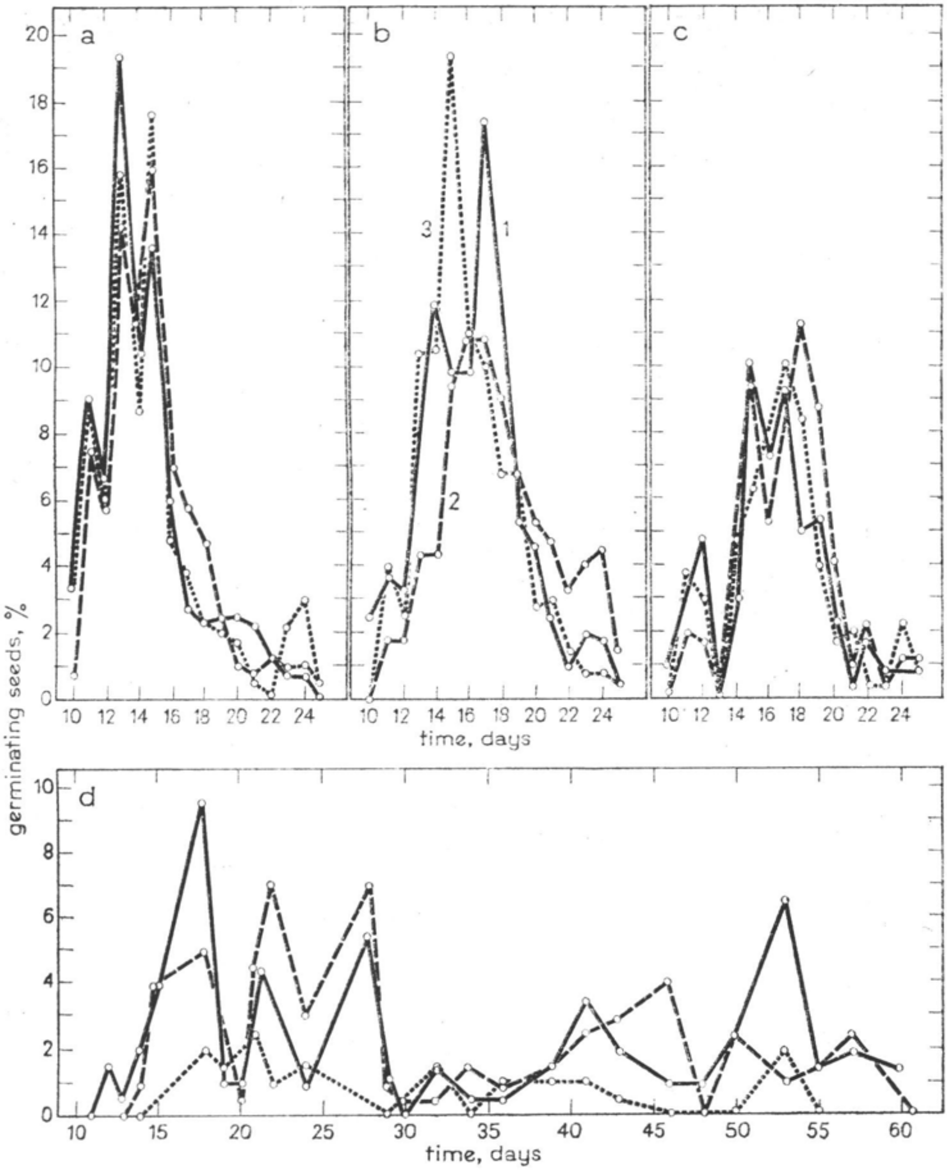

Fig. 2. Dynamics of germination of *Vaccinium myrtillus* seeds
On filter-paper: a — newly collected seeds, b — seeds kept for 3 months at 2° C, c — seeds kept for 6 months at 20° C; germination on soil originating from the same stand as the seeds: d — seeds kept for 3 months at 2° C. Curve 1 — control stand at Kokotek, 2 — not extremely polluted stand at Brynica II, 3 — extremely polluted stand at Żyglinek

The picture of survival of the seedlings germinating on filter-paper (from seeds stored for 2.5 months) and then transplanted on soil or litter taken from the same stand as the seeds, points to a clear sequence of the rates of survival, decreasing in time (Fig. 3). It is in agreement with the increasing pollution degree, lower values beeing always found for the litter than for the soil. The above mentioned sequence is disturbed only after the 90th day from planting the seedlings on the soil/litter, this being particularly visible after 102 days. The differentiation of survival seems to be very substantial, e.g. on the 80th day the rate of survival of the extremely polluted seedlings equals 35% and of the less polluted and the control ones — 72% and 79%, respectively. The prolongation of the time of storage of the seeds from 3 to 6 months does not influence essentially the survival pattern of the corresponding seeds on the soil in the period up to the 80th day. However, the scattering of the survival rates of the extremely polluted seeds was remarkable.

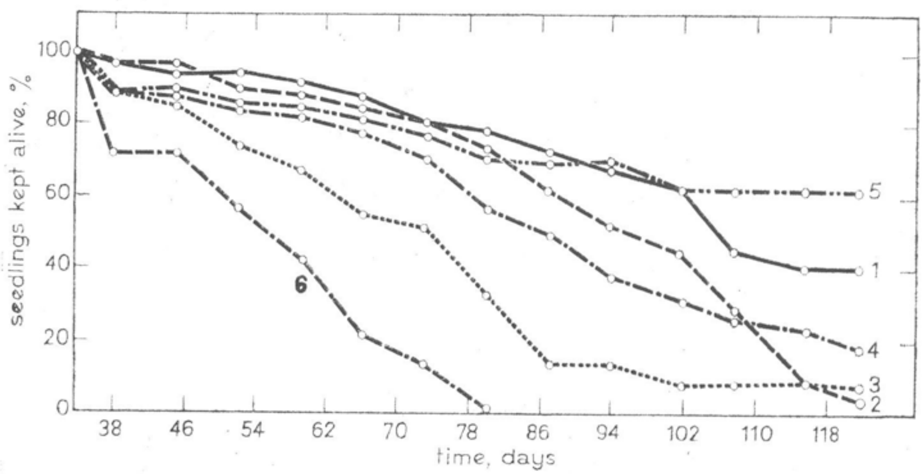

Fig. 3. Rate of survival of seedlings on soil and litter from the same stand as the seeds

Seedlings from control seeds on control soil (1) and litter (2), seedlings from less polluted seeds on less polluted soil (3) and litter (4), seedlings from extremely polluted seeds on extremely polluted soil (5) and litter (6)

Germination of seeds extremely differing in their level of pollution, carried out on soil and litter well blended mixtures, also differing widely in their degree of pollution, shows the rates of survival decreasing in the following order: C_s/C_m, Ex_s/C_m, C_s/Ex_m and Ex_s/Ex_m, where C and Ex denote, respectively, the conditions of the control and extreme pollution for the seeds (s) and the soil-litter mixture (m) (Fig. 4a). These regularities are disturbed only after the 100th day. In the case of the less polluted seeds the picture becomes complicated (Fig. 4b), their rates of survival on the soil-litter mixtures of two different levels of pollution being higher than on the unpolluted soil-mixture composition. For the latter case the rate of survival on the 96th day is 2-3 times lower than for the system polluted seeds-polluted mixture of soil and litter.

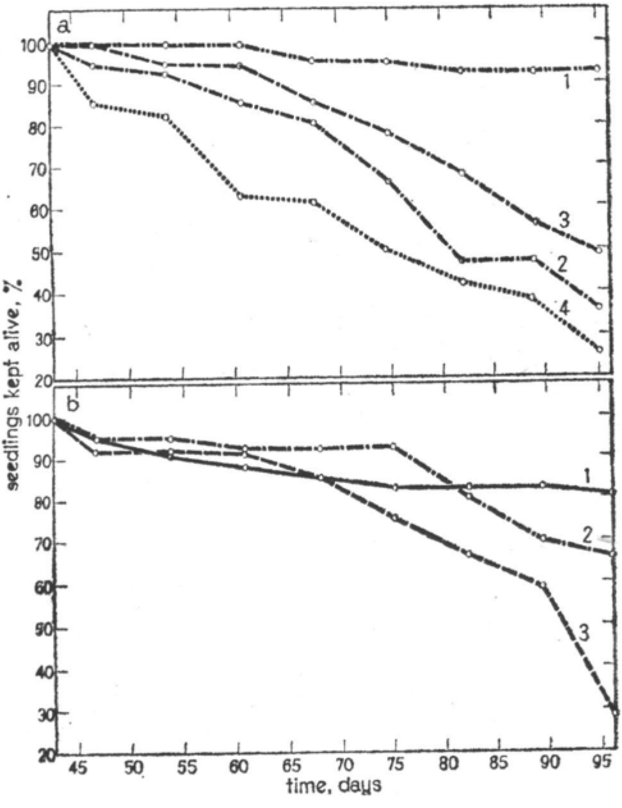

Fig. 4. Rate of survival of seedlings growing on mixtures of soil and litter of different degree of pollution

a) Control seedlings on control soil-litter mixture (1), and on extremely polluted mixture (2), seedlings from extremely polluted seeds on control mixture (3) and on extremely polluted soil-litter mixture (4). b) Seedlings from less polluted seeds on less polluted soil-litter mixture (1), on extremely polluted mixture (2) and on control mixture (3)

The data concerning the germination power and Pieper coefficients listed in Table 1, show that an increase of the pollution degree of seeds changes to a rather small extent these parameters of germination on filter-paper. For the processes occurring on soil or litter, the differences depending on the level of pollution were much higher.

Table 1

Germinating power and Pieper coefficients of *Vaccinium myrtillus* seeds

Origine of seeds (forest stand)	germination on					
	filter-paper			soil		litter
	delay in sowing after collection of seeds (months)					
	no delay	3	6	3	6	3
A. Germinating power						
Control, K	88.8	99.0	72.0	60.0	58.0	56.5
Less polluted, B II	86.5	87.2	65.0	60.0	67.5	47.0
Extremely polluted, Ż	85.8	92.0	65.3	20.0	7.0	23.5
B. Pieper coefficients						
Control, K	4.1	4.1	5.9	15.2	22.5	22.4
Less polluted, B II	3.9	4.6	5.7	15.5	19.2	19.0
Extremely polluted, Ż	4.0	4.0	5.8	17.9	27.3	28.8

DISCUSSION

From the point of view of mass exchange, seeds germinating on filter-paper represent a much simpler system than those germinating on soil or litter. The time-pattern of their germination dynamics appeared also much less complicated. It was only slightly different for seeds originating from fruits collected on the stands representing a different degree of pollution. This fact could result, among other things, from limited differences in the level of heavy metals; from among the seven investigated metals, the differences concern Pb, Cd and Mn only (Czuchajowska 1981). A few-month storage of seeds inside the fruits before the germination process begun created, however, better conditions for the appearance of changes caused by contamination. Therefore, the pictures of germination dynamics are more changed, although still in a limited extent. When the seeds are planted on soil or litter with different content of heavy metals, which is always much higher than that in seeds, the penetration into the seeds of metals is favoured. This could be the reason of the drastic change in germination dynamics. The authors do not consider possible to go at the present stage of research beyond the purely phenomenological description of these changes. However, it has to be mentioned that a similar content of organic matter in the investigated soils representing different pollution degrees does not oppose at all the accepted opinion that organic matter favours germination (Wollan et al. 1978). The different concentrations of metals, influencing the course of several processes going on in the soil, among other things also by changing the pH and buffer capacity values (Abouguendia and Redmann 1979), can change the quality and concentration of organic substances in the soil, diffusing into the germinating seeds. Also the microbial activity is quite different, bringing about several serious consequences.

The curves of survival of the seedlings prove that a decisive factor for this phenomenon is differentiation of the degree of pollution of the soil or litter but not that of the seeds, because the seedlings from the extremely polluted seeds show higher rates of survival on the control soil/litter than those from the control area grown on the extremely polluted soil/litter. The differences in metal content in the seeds originating from different areas are very much lower than those in the soil and litter samples. Nevertheless, the observed decrease of the survival rate of the extremely polluted seedlings grown on the control soil-litter composition, is much more pronounced than that in the control seedlings-control soil/litter system, indicating thereby that a significant role of contamination of seeds has to be also considered.

In the case of non-extreme pollution of soil, litter and seeds, the regularities given above do not appear; after a sufficiently long period of time, the rate of survival of the corresponding seedlings on the parent soil-litter mixture is distinctly higher than on the control soil/litter composition. This favours the opinion of the possible formation of ecotypes of *Vaccinium myrtillus* already resistant to pollution from the zinc-plant, which has affected the investigated ecosystem for more than 15 years. The authors have noticed the same behaviour of *Vaccinium vitis-idaea* (Czuchajowska and Strączek 1981), for which the phenomenon was more pronounced, probably because of the evergreen nature of that species.

Reverting to the fact described in Results, of non-shedding leaves by the laboratory-grown seedlings of *Vaccinium myrtillus*, corresponding to that found in natural stands, one could give the following explanation. Small plants, well hidden in the litter and moss, could survive quite well the winter period. Their further growth in early spring was facilitated by the absence of rhizomes, which begun to develop only when the seedlings were three or four years old. The fact that seedlings grown in the laboratory on highly polluted soils were larger than those originating from the unpolluted stands, resulted from the differentiation of germination and growth conditions in favour of the laboratory conditions, fully compensating the limitations connected with contamination of soil.

REFERENCES

Abouguendia Z. M., Redmann R. E., 1979. Germination and early seedling growth of four conifers on acidic and alkaline substrates. Forest Sci. 25: 358-360.

Czuchajowska Z., 1981. Changes in some ecophysiological parameters as a reflection of the influence of industrial emissions on a forest ecosystem — evaluation and attempt of explanation. (Habilitation Dissertation), Wydawnictwo Uniwersytetu Śląskiego, Katowice, 1981.

Czuchajowska Z., Lorek E., Strączek T., 1980. Accumulation of heavy metals in an ecosystem influenced by zinc-plant emissions. Acta Soc. Bot. Pol. 49: 339-348.

Czuchajowska Z., Strączek T., 1981. Study of germination of *Vaccinium vitis-idaea* seeds originating from natural stands differing in pollution by heavy metals. Bull. Acad. Pol. Sci., ser. biol. 29: (in print).

Koziracki L. A., Targoński P. N., 1978. Experiments on the generative propagation of wild shrubs of *Vaccinium sp.* in Polesie Żytomierskie. (in Polish). Sylwan 19: 29-33.

Wollan E., Davis R. D., Jenner S., 1978. Effects of sewage sludge on seed germination. Environ. Pollut. 17: 195-206.

Generatywne rozmnażanie się Vaccinium myrtillus w warunkach laboratoryjnych oraz wpływ na nie emisji huty cynku

Streszczenie

Badania prowadzono na nasionach *Vaccinium myrtillus* pochodzących z dwóch ekosystemów leśnych silnie skażonych emisjami huty cynku w Miasteczku Śląskim oraz ze stanowiska kontrolnego w lasach lublinieckich. Na stanowiskach skażonych gleba, ściółka, owoce i nasiona wykazywały duże zanieczyszczenie metalami ciężkimi. Nasiona bezpośrednio po zbiorze oraz po 3 i 6 miesiącach przechowywania w temperaturze 2° C kiełkowano na bibule oraz na próbkach gleby i ściółki pochodzących z wyżej wymienionych stanowisk. Stopień skażenia nasion w ograniczony sposób wpłynął na dynamikę kiełkowania na bibule, znacznie zaś bardziej zmienił dynamikę kiełkowania na glebie i ściółce (rys. 2). Zmiany siły kiełkowania oraz współczynnika Piepera przedstawione są w tabeli 1. Niezmiernie charakterystyczna okazała się przeżywalność siewek kiełkowanych początkowo na bibule, a potem przeniesionych na glebę, ściółkę lub mieszaninę gleby i ściółki (rys. 3 i 4). Po dostatecznie długim czasie, przeżywalność siewek rozwiniętych z nie ekstremalnie skażonych nasion na mieszaninie gleby i ściółki z macierzystego stanowiska okazała się wyraźnie większa niż dla tychże siewek umieszczonych w kontrolnej mieszaninie gleba-ściółka (rys. 4). Może to sugerować, że *Vaccinium myrtillus* zdolne jest wytworzyć ekotypy o zwiększonej "odporności" na emisje huty cynku, która oddziaływała na badane ekosystemy od przeszło 15 lat.

Przedstawiono także dokładny opis kiełkowania nasion *Vaccinium myrtillus* oraz rozwijania się siewek (rys. 1). Jednoroczne, dwu- i trzy-letnie siewki nie zrzucają liści zarówno w warunkach laboratoryjnych jak i naturalnych, czym różnią się od rozmnażających się wegetatywnie roślin tego gatunku.