

Phyton (Horn, Austria)	Vol. 43	Fasc. 2	307-317	29. 12. 2003
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Effect of Controlled Hydration Treatment on Quality of Aubergine Seeds Following Storage

By

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With 2 Figures

Received July 7, 2002

Accepted February 10, 2003

Key words: Aubergine, *Solanum melongena* L., seed development, controlled hydration, germination.

Summary

DEMIR I. 2003. Effect of controlled hydration treatment on quality of aubergine seeds following storage. – *Phyton* (Horn, Austria) 43 (2): 307–317, 2 figures. – English with German summary.

Aubergine (*Solanum melongena* L.) seeds were harvested 40, 45, 50, 55 and 60 DAA (days after anthesis) and 42, 45, 50, 55, 60, 70 and 80 DAA in 1999 and 2000, respectively. Seeds were stored with that 11 ± 0.2 % moisture at 25 °C for 1 year. At the end of the storage, seeds of the each lot either was hydrated (one batch) in aerated distilled water at 25 °C for 42 hours (Controlled hydration, CH) and the other batch was not (untreated). Seed quality was assessed by normal seedling percentages, mean germination time and 10th day seedling root length. CH treatment significantly increased the normal germination percentages of seeds from the earlier harvests (40, 45, 50 DAA in 1999; 42, 45 DAA in 2000) and the later harvest of 80 DAA in 2000 compared with mature lots. Mean times to germination of developmentally different seed lots were reduced by the treatment at all harvests in both years. Treated seeds in 1999 had more than 50 % longer root lengths than untreated seeds in all harvests, while in 2000, treatment rather effective on root lengths of earlier and later harvests than mature ones.

Results suggested that controlled hydration treatment was effective through the repair of seed ageing that had occurred during storage in developmentally different aubergine seed lots.

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Zusammenfassung

DEMIR I. 2003. Einfluss kontrollierter Feuchtebehandlung auf die Qualität von gelagerten Auberginensamen. – *Phyton* (Horn, Austria) 43 (2): 307–317, 2 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Samen von Melanzanipflanzen (*Solanum melongena* L.) wurden im Jahr 1999 am Tag 40, 45, 50, 55 und 60 nach der Anthese und in der Saison 2000 am Tag 42, 45, 50, 55, 60, 70 und 80 nach der Anthese geerntet. Die Samen wurden ein Jahr lang bei $11 \pm 0.2\%$ Feuchtigkeit und $25\text{ }^{\circ}\text{C}$ gelagert. Nach der Lagerung wurden Samen jeder Gruppe in belüftetem, destilliertem Wasser bei $25\text{ }^{\circ}\text{C}$ 48 h lang gequollen (kontrollierte Quellungsbehandlung). Als Kontrolle blieben Samen jeder Gruppe unbehandelt. Die Samenqualität wurde durch die Bestimmung des Anteils normal entwickelter Keimlinge, der Keimungsrate, der durchschnittlichen Zeit bis zur Keimung sowie der Keimwurzellänge an 10 Tage alten Keimlingen bewertet. Die kontrollierte Quellung erhöhte den Anteil normalentwickelter Keimlinge aus Samen der frühen Ernten (bis 50 Tage nach der Anthese in 1999 und bis 45 2000) sowie der späten Ernte (80 Tage im Jahr 2000). Die mittlere Keimungsdauer wurde durch die Quellungsbehandlung bei Samen aller Sammelzeitpunkte verkürzt. Die Keimwurzellänge wurde bei allen im Jahr 1999 um mehr als 50 % verlängert im Vergleich mit unbehandelten Samen verlängert, während sich die Behandlung im Jahr 2000 die Wurzellänge eher bei Keimlingen aus früh oder spät geernteten Samen als bei reifen auswirkte.

Die Ergebnisse weisen darauf hin, dass die kontrollierte Quellungsbehandlung durch Reparatur der Alterungseffekte, die während der Lagerung von in unterschiedlichen Reifungsstadien auftraten, wirksam war.

Introduction

Seed ageing in storage depends on both pre-storage (environment, water stress etc.) and post-storage (seed moisture, storage temperature and oxygen) factors as well as the developmental stage of the seed (ELLIS & ROBERTS 1980). Maturation stage of the seed is one of the important pre-storage factors that influences seed survival in storage (PRIESTLEY 1986). Previous studies on relationship between seed development and storage longevity tests (K_1 , p_{50}) showed that less mature and overmature seed lots lose viability earlier than fully mature ones in a number of crops (ZANAKIS & al. 1994, SANHEWE & ELLIS 1996, DEMIR & SAMIT 2001). Recently, similar conclusions were also drawn in aubergine. DEMIR & al. 2002 found that aubergine seeds harvested between 55 and 70 DAA had higher potential seed longevity (K_1) than those of earlier and later harvests. In continuously flowering vegetable species, such as aubergine, seed lots produced following once-over mechanical harvesting are likely to contain seeds having a range of maturity within the same batch. As a result, a seed lot that contains a higher proportion of immature and overmature seeds might have shorter longevity since these seeds lose viability faster.

A range of treatments to improve the germination performance have been developed over the last 20 years (HEYDECKER & COOLBEAR 1977). These

are commonly referred to priming and involve hydration by using various osmotic solutions such as PEG (Polyethylene Glycol), inorganic salts and mannitol. During hydration, metabolic activity proceeds, but the radicle does not emerge. Recently, controlled hydration treatments in which seeds were incubated in water for specific times and at a preuse temperature have been developed (GRAY & al. 1990, BASU 1994, THORNTON & POWELL 1995). In such treatments, radicle protrusion can be prevented by manipulating the time or temperature of the treatment rather than the osmoticum (BURGASS & POWELL 1984). Controlled hydration treatments have been used to improve the viability and rate of germination of seeds of various crops in optimum and adverse conditions (SAVINO & al. 1979, FUJIKURA & al. 1993, BASU 1994, THORNTON & POWELL 1995). One of the main advantages of the treatment is the extension of storage longevity possibly through the repair of seed ageing (HOFFMANN & STEINER 1994, POWELL & al. 2000, DEMIR 2002). POWELL & al. 2000 found that improvement in quality obtained by the treatment was higher in a low quality, aged cauliflower seed lot than in a high quality, unaged lot. They concluded that this could be explained by induction of metabolic repair by the treatment in low quality seeds.

Species such as aubergine, in which seed lots of seeds having wide range of maturities may therefore benefit from a hydration treatment after storage. This would have practical value in that seed producers would be able to upgrade carry-over seed following storage. The aims of the present work on aubergine were (1) to determine the effect of the controlled hydration treatment on seed quality after one year of storage at 25 °C with 11 ± 0.2 % moisture content (mild environment); and (2) to observe how serially harvested (developmentally different) seed lots respond to the treatment in terms of quality improvement.

Materials and Methods

Seed harvest

The detailed cultivation of plants of aubergine, cultivar Pala (*Solanum melongena* L.), between May and October in the year 1999 and 2000 was described in DEMIR & al. 2002. Three hundred flowers at full anthesis were tagged in each year and fruits were harvested 40, 45, 50, 55 and 60 days after anthesis (DAA) 1999 and 42, 45, 50, 55, 60, 70 and 80 DAA in 2000. Fruits were crushed and seeds were extracted. Moisture contents of seeds were determined by the low temperature oven method (ISTA 1996). Mean seed dry weight was determined on four replicates of one hundred seeds of each harvest after drying at 130 °C for 1 hour. Dried seeds were tested for germination on top of moist filter papers (Filtrak, GmbH, Baerenstein, Germany) in Petri dishes (9 cm) at 25 °C for 14 days. Each test comprised of four replicates of 50 seeds from each harvest and seedlings were classified as normal and abnormal according to ISTA 1996. Remaining seeds were then kept with 8 % moisture content in sealed glass jars.

Seed storage

Eight hundred seeds were stored at 25 ± 1 °C with 11 ± 0.2 moisture content for one year. Moisture contents were equilibrated to about 11 % by keeping the seeds on top of mash trays above saturated NaCl at 20 °C for 15 days in a desiccator. Eight hundred seeds of each harvest were then sealed in laminated aluminium foil bags and placed in an incubator at 25 °C for one year from November 2000 to November 2001. Seeds were then treated by controlled hydration treatment as described below or left untreated.

Controlled hydration treatment

Controlled hydration (CH) was completed for half the stored seeds (i.e. 400 seeds). The remaining stored seeds were untreated. The procedure for controlled hydration treatment has been described in DEMIR 2002. Briefly, CH was carried out in glass tubes (11 cm height and 3 cm diameter) containing distilled water and aerated by an aquarium pump. The glass tubes were placed in a tube holder in a water bath at 25 °C for 42 h in the dark. Four hundred seeds with 50 ml of distilled water were put in each glass tube for each harvest. Each CH tube was aerated by a syringe and all syringes were connected through plastic pipes to an aquarium pump that supplied at a rate of 1 L min^{-1} at 25 °C. Following hydration treatment, seeds were dried for 48 h at 20 ± 2 °C to their original moisture content (8 %).

Seed germination, mean germination time and root length tests

Germination tests of treated and untreated seeds of each lot were set up with four replicates of 50 seeds between filter papers (10×10 cm) (Filtrak, GmbH, Baerenstein, Germany) at 25 °C for 14 days in the dark (ISTA 1996). Each filter paper was moistened with 6 ml of distilled water and was put into plastic bags in order to prevent water loss. Germination counts (2 mm radicle protrusion) were determined daily. Normal seedlings (well developed root and shoot) were assessed according to ISTA rules (1996) at the end of the test (14 day). Mean germination time was calculated as follows (ELLIS & ROBERTS 1980):

$$\text{MGT} : \frac{\sum n \times D}{\sum n}$$

where, MGT is the mean germination time, n is the number of seeds newly germinated at hours and D is the number of hours from the start of germination.

The length of the roots of all normal germinated seedlings were measured 10 days after the germination tests were set up and means were taken as mm/plant.

Means of germination and root length were subjected to analysis of variance (ANOVA, $P=0.05$) by using MSUSTAT (Oregon State University U.S.A.). Percentages were angular transformed before analysis but actual germination values are presented in the figures.

Results

Seed moisture contents at harvest were higher than 40 % in all samples and both years but declined to 8 ± 0.3 % after drying (Table 1). Seed dry weight reached a maximum of 3.39 mg at 45 days after anthesis (DAA) in 1999 and 3.68 mg at 42 DAA in 2000 and remained stable thereafter. In both years the first harvest produced seeds having a total germination around 60 % (Table 1), with normal germination of 51 % 1999 and 41 % 2000. All

Table 1. Changes in seed moisture content at harvest, after drying and in storage, total and normal germination percentages and seed dry weight of aubergine seed lots during development in 1999 and 2000.

DAA	(Year)	Seed Moisture (%)			Total/normal	Seed dry weight (mg/seed)
		at harvest	after drying	at storage		
40	1999	51.4	8.1	11.2	59/51	3.23
45		50.8	8.0	11.0	92/68	3.39
50		45.8	7.8	10.9	97/79	3.40
55		48.9	8.3	11.2	98/86	3.40
60		48.1	7.7	10.9	95/87	3.43
42	2000	44.8	7.9	11.2	63/41	3.68
45		41.8	8.2	11.1	94/78	3.64
50		40.2	8.1	10.8	92/91	3.69
55		43.0	7.8	10.9	99/93	3.71
60		44.1	8.3	11.0	99/92	3.73
70		43.3	8.2	11.1	99/94	3.66
80		42.6	8.2	11.0	98/90	3.65

DAA : Days After Anthesis.

other harvests produced seeds with >90 % total germination, with maximum at 55 DAA in 1999 and 55–70 DAA in 2000. Normal germination reached a maximum at 55–60 DAA (1999) and 50–80 DAA in 2000.

The equilibration of seeds at 72 % relative humidity resulted in seed moisture contents between 10.8 % and 11.2 % (Table 1). Storage at this moisture content and 25 °C for one year resulted in a reduction in the normal germination of seeds from all harvest dates in 1999 with a greater fall seen for the earlier harvests (Fig. 1). Controlled hydration treatment of stored seeds resulted in increased normal germination of seeds from all 1999 harvest dates. The increase was greater and significant for the earlier harvest dates (40–55 DAA). In the earlier seed samples at 40, 45 and 50 DAA the improvements obtained following controlled hydration were 15, 18 and 12 %, respectively. Differences were comparatively lower at 55 and 60 DAA being 5 and 3 % only (Fig. 1). In both the control and treated seeds, normal germination gradually increased from 40 to 55 DAA when they reached to 73 and 78 %, respectively.

Similar changes in germination during storage and following CH treatment were observed after the 2000 harvests, although the changes were not as striking as after the 1999 harvests. Germination again fell during storage, with a greater fall following the early harvests, but this decrease in germination was less than for the 1999 seeds. Controlled hydration resulted in increased germination of the stored seeds, but in this case the increase was seen only in the seeds from the two earliest (42, 45 DAA) and latest (80 DAA) harvest dates (Fig. 2).

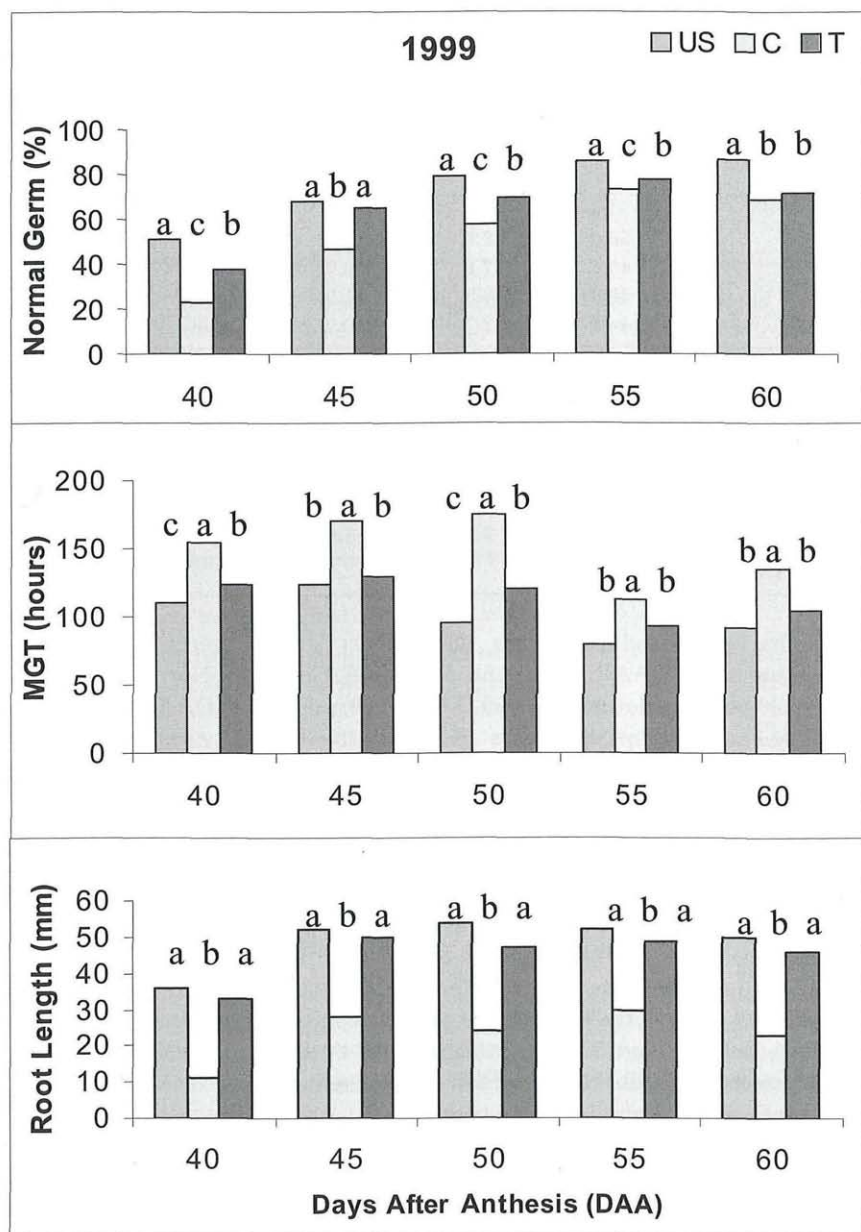


Fig. 1. Changes in normal germination percentages, mean germination time (hours) and root length (mm) of serially harvested aubergine seeds in 1999 unstored (US), following storage at 25 (C with 11 ± 0.2 % for 1 year (C) and stored and treated (T) with controlled hydration. Means with different letters are significantly different ($P = 0.05$) at each harvest.

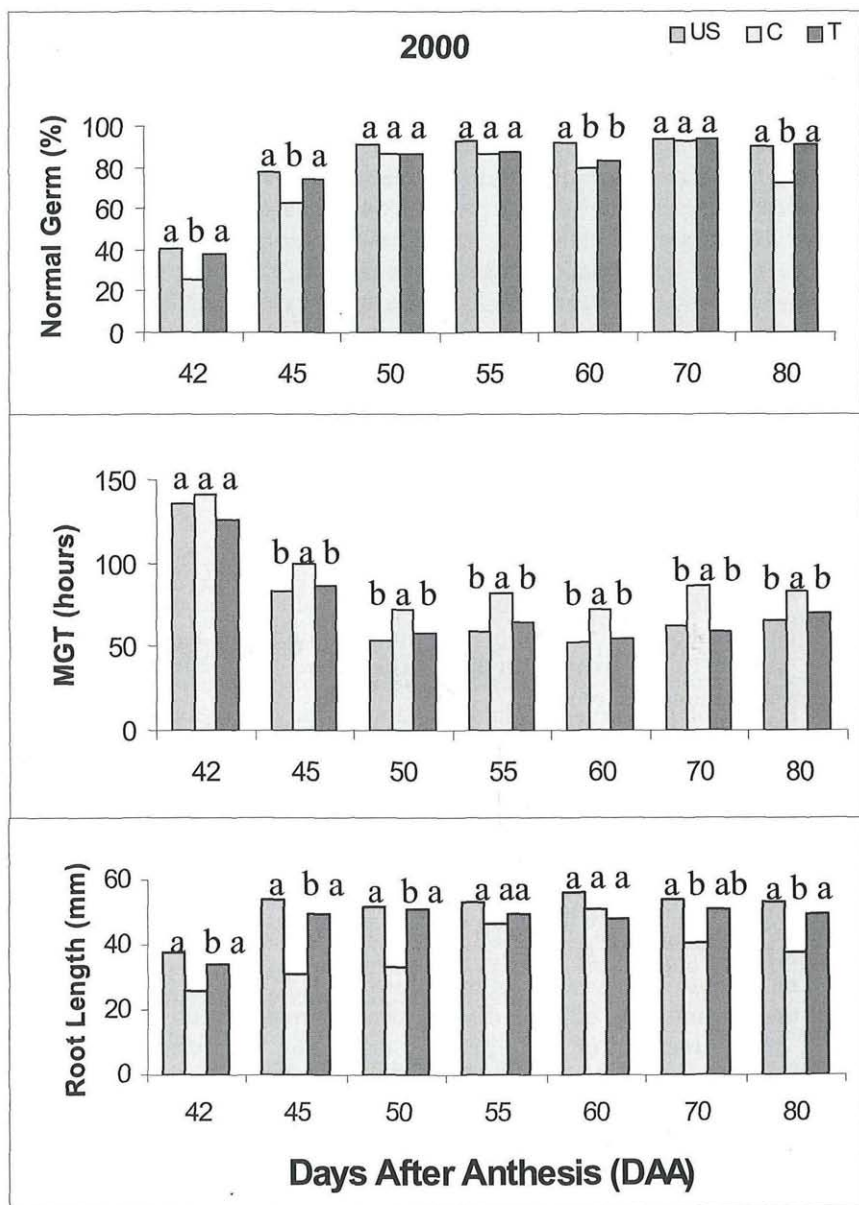


Fig. 2. Changes in normal germination percentages, mean germination time (hours) and root length (mm) of serially harvested aubergine seeds in 2000, unstored (US), following storage at 25 (C with 11 ± 0.2 % for 1 year (C) and stored and treated (T) with controlled hydration. Means with different letters are significantly different ($P = 0.05$) at each harvest.

Storage of seeds at 25 °C with 72 relative humidity for one year increased mean germination time, i.e. decrease in germination rate, significantly at all harvests in both years (Figs. 1, 2) with the exception of 42 DAA of 2000 (Fig. 2). However, mean germination time of all stored seed samples after CH was reduced to a similar level to that of unstored seeds. In 1999, the decrease in germination time after CH, was more prominent for seeds from harvests at 40, 45 and 50 DAA than those at 55 and 60 DAA as seen in the germination data. The effect of harvest date on response to post-storage CH treatment was less clear in 2000 (Fig. 2).

Similar effects of CH treatment were seen in the root length measurements. Thus, root length decreased following storage, but was restored by CH to a comparable value to that of unstored seeds. The effect of storage and subsequent CH treatment was significant at all harvest dates in 1999 (Fig. 1) and at early (42, 45, 50 DAA) and late (70, 80 DAA) harvests in 2000 (Fig. 2).

Discussion

Controlled hydration treatment following a period of one year storage at 25 °C resulted in improved aubergine seed quality as assessed by normal germination, mean germination time and seedling root length. Improvement tended to be greater for seeds that were harvested immature or overmature than mature seeds.

Immature seeds (indicated by dry weight) tended to show reduced seed longevity, seen in a greater decrease in normal germination, mean germination time and root length after storage compared with mature seed. This agrees with previous work on seed longevity in relation to seed development indicated in a number of crops such as soybean (ZANAKIS & al. 1994), bean (SANHEWE & ELLIS 1996), tomato (DEMIR & SAMIT 2001) and aubergine (DEMIR & al. 2002), that the more mature seeds had the longer survival in storage than immature ones.

The greater improvement of immature seeds following CH may result from the continuation of their developmental processes during CH treatment, or, the removal of some primary dormancy. This was proposed in previous work in which osmotic priming using PEG or salt solutions advanced the development of immature vegetable seeds such as carrot (*Daucus carota* L.), celery (*Apium graveolens* L.), muskmelon (*Cucumis melo* L.) and broccoli (*Brassica oleraceae* L. var. *italica*) seeds. Advancement was seen rather on germination rate than germination percentages (VAN DER TOORN 1989, WELBAUM & BRADFORD 1991, JETT & WELBAUM 1996).

However, another of the reported benefits of the hydration treatments is to repair damage incurred during storage (RAO & al. 1987, HOFMAN & STEINER 1994, POWELL & al. 2000). Enhanced protein and RNA synthesis were observed during treatment in a number of species (KHAN 1992). Be-

sides, observations on embryo enlargement (HEGARTY 1970), biochemical evidence such as increase in DNA replication and β -tubulin synthesis (POWELL & al. 2000), increase in some enzyme activities and protein structures (SMITH & COBB 1992), confirmed that treatment leads to initiation of the reactions involved in the repair of ageing. Moreover, BASU & PAL 1979 found that hydration can also have effect on maintenance of viability by leaching toxic substances from the seed and anti-pathogenic action. VILLIERS & EDGE CUMBE 1975 reported that enzymatic repair mechanisms became operative in fully imbibed lettuce seeds. CH in this work improved normal germination, mean germination time and root length following one year of storage which provides evidence to support the occurrence of repair mechanisms during CH treatment.

When controlled hydration was compared with some other priming methods such as inorganic salt solutions, it can be proposed that it is less expensive, easy, practical and promising for developing countries (BASU 1994). However, optimum treatment temperature and period for different species should be determined if hydration is to be considered in practice for other species and large quantities of seeds.

Improving the quality of aged aubergine seeds during development after CH treatment obviously gives an opportunity (1) to upgrade seed quality through repair of ageing and therefore to use carry over seeds in the following season (2) to increase the uniformity of germination and rapid seedling production in seed lots in which differentially developed seeds exist in the same lot. The present work showed that this can be achieved through improving the quality of the less or over mature portions of seeds within the same lot. The seed storage environment in this study was based on a Mediterranean climate in which the yearly mean temperature and relative humidity vary about 20–25 °C and 70–80 %, respectively and in which aubergine seeds lose quality within two years (PASSAM & al. 1999) Hydration treatments have potential to repair the seed ageing that occurs during storage in such an environment and can be applied to extend the use of seeds in the following production year.

Acknowledgement

I express my gratitude K. MAVI for allowing me to use aubergine seeds left from his study.

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Phyton (Horn, Austria) 43 (2): 317–318 (2003)

Recensiones

HÖRANDL Elvira, FLORINETH Florin & HADACEK Franz 2002. Weiden in Österreich und angrenzenden Gebieten. – Gr. 8°, 164 Seiten, 70 Abb.; kart. – Arbeitsbereich Ingenieurbiologie und Landschaftsbau, Institut für Landschaftsplanung und Ingenieurbiologie, Universität für Bodenkultur, Wien. – € 29,-. – ISBN 3-9501700-0-6.

Der ansprechende Band beginnt mit allgemeinen Abschnitten: Systematik und Evolution der Gattung (p. 7–16), Standortsökologie und Verbreitung (p. 17–20), Weidenspezifische Inhaltsstoffe (so auf p. 21!) (p. 21–28), Pilze, Bakterien und Insekten auf Weiden (p. 29–32), Die Verwendung von Weiden (p. 33–46). Das Inhaltsstoffkapitel gilt vor allem Salicin und Flavonoiden, bei Verwendung stehen Austriebsvermögen und Bodenbefestigungen im Vordergrund. Der sechste Abschnitt (p. 47–78) gilt den Unterscheidungsmerkmalen und enthält vier Bestimmungsschlüssel (für beblätterte Zweige, solche mit weiblichen und männlichen Kätzchen und für Knospen und Zweige im Winterzustand) für die 32 Arten (ohne Hybriden). Anschließend sind die 32 Arten behandelt; der Text ist jeweils un-

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Zeitschrift/Journal: [Phyton, Annales Rei Botanicae, Horn](#)

Jahr/Year: 2003

Band/Volume: [43_2](#)

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Artikel/Article: [Effect of Controlled Hydration Treatment on Quality of Aubergine Seeds Following Storage. 307-317](#)