PHYTON

ANNALES REI BOTANICAE

VOL. 38, FASC. 2 PAG. 225–336 29. 12. 1998

Phyton (Horn, Austria)	**Vol. 38**	Fasc. 2	225–237	29. 12. 1998

Determination of Seed Vitality by High Frequency Electrophotography

By

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With 5 Figures

Received December 12, 1997

Accepted May 27, 1998

Key words: Seed, vitality of seed, high frequency electrophotography.

Summary

ČATER M. & BATIČ F. 1998. Determination of seed vitality by high frequency electrophotography. – Phyton (Horn, Austria) 38 (2): 225–237, 5 figures. – English with German summary.

The vitality of seeds may be tested by various methods, but the negative side of almost all methods is their relatively long duration and harmful effects on the tested seeds. By applying high frequency electrophotography to seed vitality tests the testing time can be much reduced and the vitality of the seeds / fruits preserved. The fruits of common beech (*Fagus sylvatica* L.) and common maple (*Acer pseudoplatanus* L.) were exposed to a high frequency electromagnetic field for a short time and

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photographed. On the photographs the amplified electric potential of living seeds was obtained and showed the vitality of the seeds. A comparison between two methods of testing seed vitality – high frequency electrophotography (HFEP) and the commonly used TTC method was made; both showed similar results. The advantages of the high frequency method are its shorter duration, lower price and undamaged seed.

Zusammenfassung

ČATER M. & BATIČ F. 1998. Bestimmung der Vitalität von Samen mittels der Hochfrequenzelektrophotographie. – Phyton (Horn, Austria) 38 (2): 225–237, 5 Figuren. – Englisch mit deutscher Zusammenfassung.

Die Vitalität von Samen kann mit verschiedenen Methoden festgestellt werden, nachteilig ist aber für beinahe alle Methoden, daß sie relativ lange dauern und schädlich für die getesteten Samen sind. Durch die Anwendung der Hochfrequenzelektrophotographie zur Vitalitätsprüfung von Samen kann die Prüfzeit stark verkürzt und die Lebensfähigkeit von Samen/Früchten erhalten bleiben. Die Früchte der Buche (*Fagus sylvatica* L.) und Ahorn (*Acer pseudoplatanus* L.) wurden einem hochfrequenten elektromagnetischem Feld für kurze Zeit ausgesetzt und photographiert. Auf den Photos wurde das vergrößerte elektrische Potential von lebenden Zellen erhalten, welches die Vitalität von Samen widergibt. Zum Vergleich wurden zwei Methoden zur Vitalitätsprüfung von Samen angewandt: die Hochfrequenzphotographie (HFEP) und die für gewöhnlich verwendete TTC-Methode; beide zeigten ähnliche Ergebnisse. Die Vorteile der Hochfrequenzphotographie sind kürzere Dauer, der geringere Preis und die unbeschädigten Samen.

Introduction

Storing the seeds of forest trees plays an important role in ensuring a supply of seedlings for reforestation in the years without fructification. Nowadays storing of seeds is important for the protection of natural genetic diversity and especially in cases of natural imbalances, when natural regeneration is in question.

In forestry and agriculture a variety of techniques and approaches are used for evaluation and estimation of seed vitality. The most common are biochemical methods, known to be very reliable (ISTA 1993). Vitality can also be tested by mechanical or radiographic tests and by the classical germination test. All of these tests are specific, adapted to the characteristics of species and therefore standardised. After passing the test the seeds are destroyed; the only exception is the germination test, where a longer period of time is necessary to obtain results and also dormancy must be considered.

Assessment of seeds by high frequency electrophotography enables the more rapid testing of seeds. In this method seeds are exposed to an electromagnetic field for a short time and photographed; on the photographs traces of the amplified electromagnetic potential of the living seed can be observed. After passing the test the seeds are unchanged and alive; they

can be tested again to control the vitality of the same seeds after a certain period of time. The method has proved to be successful with the seeds of maize (PLIBERŠEK 1990).

In order to evaluate the possible use of the high frequency method in testing the vitality of forest tree seeds, we carried out study on the seeds / fruits of common beech and common maple.

Material and Methods

High frequency electrophotography

High frequency electrophotography is more often known as Kirlian photography (LEBAR 1975, JOHNSON 1977). Many theories and assumptions have been made, because the method is difficult to quantify and repeat because many factors affect the discharge (JOHNSON 1977).

From the electrotechnical point of view discharges are present when an electrical current passes through an isulating substance (ENGEL 1993, MCGRAW-HILL 1994). Between two electrodes a strong electromagnetic field is created and ionised gases become electrically conductive. Molecules of gases transit from higher to lower energetic states and photons of characteristic energies are emitted. Traces called streamers – the edges of electronic avalanches are created – and may be observed. The phenomenon is similar to electric welding, where a pulsating discharge is present. The type and the speed of the discharge depends on the distance between the electrodes and the pressure of the gas (LEBAR 1975).

If an object is placed in that field – in our case a seed – we can observe formation of a characteristic ring around the seed, called a corona , which is one of several types of discharge. A corona is discharge between two electrodes, where one or both have very little diameter, so the intensity of the electrical field near the electrode is significantly higher than elsewhere. (ENGEL 1993, MCGRAW-HILL 1994).

Important discoveries in this field were achieved by N. TESLA and later by S. and V. KIRLIAN, who dedicated 25 years to research work before first publishing in 1961 (KIRLIAN & KIRLIAN 1961).

In 1989 at Leigh University, USA, a special method for discovering changes and anomalies in the structure of materials was developed. The method is fast, cheap and reaches the accuracy of NMR (nuclear magnetic resonance). Anomalies in the structure of a material are shown in changes by the discharges; it is useful for discovering microscopic cracks on aeroplane and ship constructions (LERNER 1989).

There have been many other interesting discoveries in connection with corona discharge photography, such as detecting cancer (GRIFF & al. 1983), or detecting anomalies on compact discs (LIGHTWOOD 1994). In spite of such facts it is wise to treat the results and discoveries of this field with a certain measure of caution.

Photographing seeds

Photographing seeds was performed by a device made by D. OGRINC of Slovenska Bistrica, Slovenia; with it we can observe electrical discharges from objects with dimensions below 20 cm × 25 cm. The device is connected to main voltage 220 V (50 Hz); by pressing a starter a discharge is triggered. The frequency of the discharge could be set from 30 to 5400 Hz and time of exposure from 0 to 6 seconds; the object must be connected to a mobile electrode for observation, as shown in Fig. 1.

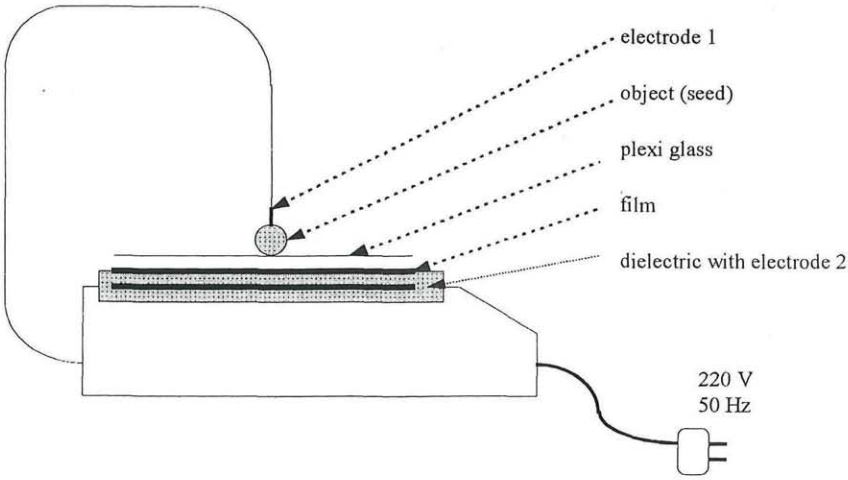

Fig.1: Scheme of HF device

When a film is placed under the object, light is noticed on the film by discharge, which can be quantified. Photographing was performed by placing photographic paper (B&W PH/Forte) under the object. After exposure the developed pictures represent negative images, which are usually easier to evaluate than positives.

When photographing, a frequency of 3000 s^{-1} was used; the time of exposure was set before every series, so that the maximal area of corona was established. The time for developing photos was precisely controled for all photos in all series (60 s).

First a pilot sample of 200 seeds from common beech (*Fagus sylvatica* L.) and common maple (*Acer pseudoplatanus* L.) were taken to establish a limit value of the vitality coefficient »f«. Seeds were first photographed at discharge and later subjected to the TTC test.

A sample of randomly chosen 120 seeds from common beech and 120 seeds from common maple followed to test the vitality of seeds. It was possible to make only one photo of a seed discharge at the time. Results from photographed seeds were also compared with the biochemical test for vitality by the tetrazolium (TTC) method on the same seeds.

Later a sample of 200 common maple seeds followed and discharge results were compared with germination tests on the same (live) seeds.

Photographing took place in the darkroom, so results were instant. Because of several factors influencing the discharge, seeds were photographed in series of 20, to establish as homogenous circumstances as possible.

The sample of seeds was random, obtained from the Semesadike Mengeš Nursery, Slovenia. Photographs and all other tests were made at Slovenian Forestry Institute.

Vitality of seeds determined by the tetrazolium (TTC) test

When testing common beech and common maple seeds a standard procedure was used. The biochemical method with tetrazolium is based on the fact that living tissues of seeds / embryos react with the testing solution; due to reduction processes in living cells TTC solution changes on contacting living tissues to an intense red and

becomes insoluble in water. After passing the TTC test the seeds are dead. The time of soaking the seeds in water (18 hours for both *Acer* and *Fagus*), preparation and then soaking in TTC solution (24 hours) is specific and characteristic for every species.

After soaking in TTC solution for the prescribed time, seeds were cut and divided into into viable and non-viable categories (ISTA 1993) according to the location and intensity of colouring in the tissues (REGENT 1980).

Results

Vitality of photographed seeds

For evaluation of photographs a coefficient of vitality "f" was derived, which was calculated by using the ratio between the projected area of the seed and the whole area of the corona, so results were comparable no matter what was the size of the seed (Fig. 2). In the case of vital seeds this coefficient was low, according to greater relative area taken by the corona than by the seed. In the case of non-living or less vital seeds, the coefficient was higher.

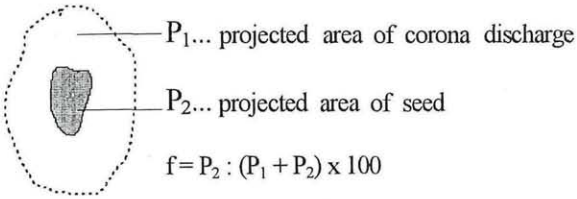

Fig.2: Relation between area of corona and seed as vitality coefficient

The assumption was made that a higher value of the coefficient "f" is characteristic of non-vital seeds, so a negative result from the biochemical test with tetrazolium should confirm this statement.

The test showed that a value of 20,0 for the coefficient "f" separated vital from non-vital seeds. It means that the seed was vital if the projection of the seed compared to the whole area of discharge was 20% or smaller.

Comparison of the vitality coefficient "f" with the biochemical vitality test based on tetrazolium showed agreement in the case of beech and maple seeds. The results are presented in Tables 1 and 2. The area of the corona of non-vital seeds was smaller compared to vital seeds and therefore the vitality coefficient was higher. The difference was presumably caused by a higher electromagnetic or electrochemical potential, which is stronger in the case of living seeds. The electrochemical potential – strongly connected with living processes – is less expressed or even absent in non-living seeds; we can say that by photographing the electrical discharge the living potential of seeds can be registered.

Data within each series were divided according to the vitality determined by TTC and the number of seeds with an "f" value above the

Table 1:

Vitality coefficient »f« and vitality test (TTC) for common beech and common maple seeds

COMMON BEECH SEEDS												
series	I		II		III		IV		V		VI	
TEST N°	f	TTC	f	TTC	f	TTC	f	TTC	f	TTC	f	TTC
1	8	+	19	+	16	+	11	+	37	-	20	+
2	11	+	24	+	25	+	25	+	17	+	28	-
3	18	+	19	+	13	+	18	+	24	-	17	+
4	8	+	23	+	18	+	19	-	30	-	22	-
5	8	+	64	-	14	+	14	+	22	-	16	+
6	18	+	19	+	14	+	17	+	15	+	34	-
7	20	+	20	+	15	+	18	-	27	-	26	-
8	10	+	19	+	11	+	14	+	26	-	29	-
9	14	+	18	+	16	+	21	-	27	-	13	+
10	15	+	16	+	15	+	21	-	13	+	26	-
11	9	+	34	-	19	+	11	-	29	-	22	-
12	20	-	20	+	15	+	24	-	31	-	19	+
13	15	+	18	+	16	+	22	-	13	+	19	+
14	15	+	17	+	30	+	21	-	20	-	32	-
15	24	-	50	-	12	+	29	-	12	+	12	+
16	12	+	14	+	10	+	15	+	22	-	32	-
17	12	+	14	+	17	+	32	-	28	-	25	-
18	27	-	15	+	18	+	21	-	18	+	13	+
19	10	+	9	+	11	+	35	-	11	+	19	+
20	12	+	37	-	35	-	12	+	30	-	21	-

COMMON MAPLE SEEDS												
series	I		II		III		IV		V		VI	
TEST N°	f	TTC	f	TTC	f	TTC	f	TTC	f	TTC	f	TTC
1	10	+	15	+	11	+	26	-	20	+	19	+
2	10	+	13	+	10	+	20	+	25	+	26	+
3	14	+	31	-	8	+	17	+	22	+	15	+
4	12	+	11	+	11	+	20	+	21	+	21	+
5	10	+	10	+	9	+	24	+	27	+	24	-
6	15	+	11	+	8	+	21	+	26	+	24	+
7	12	+	14	+	14	+	30	-	23	+	28	+
8	10	+	15	+	13	+	20	+	27	-	20	+
9	15	+	15	+	8	+	46	-	20	+	28	+
10	18	-	15	+	9	+	36	-	29	-	29	-
11	14	+	12	+	12	+	22	+	27	+	20	+
12	13	+	12	+	18	-	24	+	27	+	35	-
13	9	+	27	-	10	+	28	+	16	+	29	+
14	17	+	23	-	9	+	32	-	24	+	20	+
15	15	+	24	-	12	+	18	+	30	-	21	+
16	9	+	12	+	9	+	16	+	20	+	22	+
17	16	-	11	+	29	-	21	+	23	+	19	+
18	21	+	9	+	9	+	18	+	36	-	15	+
19	10	+	10	+	8	+	22	+	26	+	33	-
20	11	+	10	+	12	+	28	+	17	+	16	+

Legend: f... vitality coefficient determined after photographing seeds
TTC –... dead seed according to TTC test
+... live seed according to TTC test

Table 2

Pearson contingency coefficient (CC) and X^2 significance test

	common beech					
series	**I.**	**II.**	**III.**	**IV.**	**V.**	**VI.**
X^2	14,1***	7,5**	5,96*	7,5**	20,0***	16,3***
cc	0,64	0,52	0,48	0,52	0,71	0,67

	common maple					
series	**I.**	**II.**	**III.**	**IV.**	**V.**	**VI.**
X^2	5,9*	20,0***	9,5**	1,7	0,6	4,6*
cc	0,48	0,71	0,57	0,28	0,17	0,43

Significance: 5%... *
1%... **
0,1%... ***

	vital seeds (TTC)	non vital seeds (TTC)	S
N° of seeds above chosen "f"	a	b	a + b
N° of seeds below chosen "f"	c	d	c + d
S	a + c	b + d	n

a, b, c, d ... observed frequencies

Fig. 3: Scheme for the X^2 test

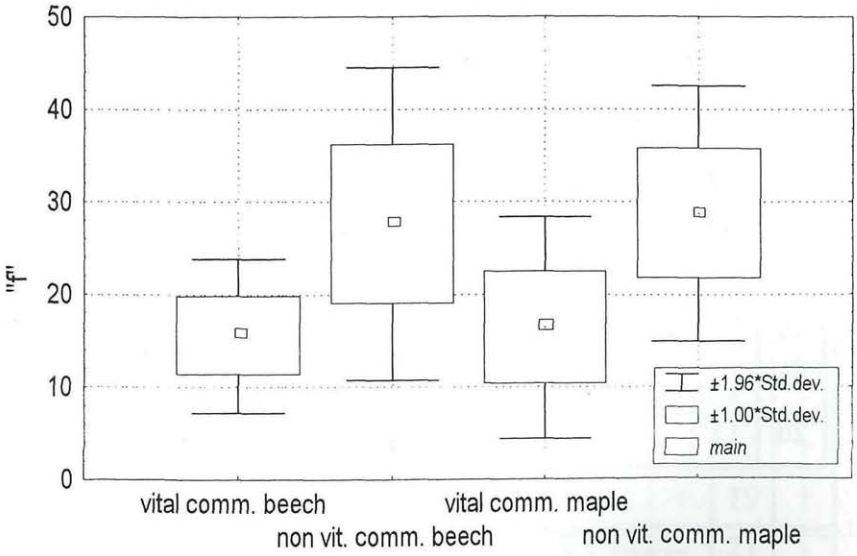

Fig. 4: Vitality coefficient for common beech and common maple seeds

chosen vitality coefficient "f", which was calculated according to the previously described formula in Fig. 3.

For the test of significance the X^2 test was used (SNEDECOR & COCHRAN 1980).

Dependence between the two attributive parameters was given by the Pearson contingency coefficient (cc) (SNEDECOR & COCHRAN 1980).

For the purpose of further statistical analysis vital seeds have been given a value 1 instead of "+" and non vital seeds a value 0 instead of "–". Vitality coefficients are presented in Tables 1 and 3 for common beech and common maple seeds (Fig. 4).

In spite of separating value 20,0 of the vitality coefficient "f", its actual values changed from series to series. The reason for disagreement might be sought in factors upon which we had no influence like air pressure, humidity, presence of ozone, change in voltage or others.

Germination of photographed seeds

Comparison of the results from electrophotography with those of germination tests was performed in the same way as it was in the case of the vitality of seeds. Tables 3 and 4 present results of the experiment with the corresponding vitality coefficients. An assumption was made that the factors which caused discordance in the results of the vitality test were the same as in the germination test (Fig. 5).

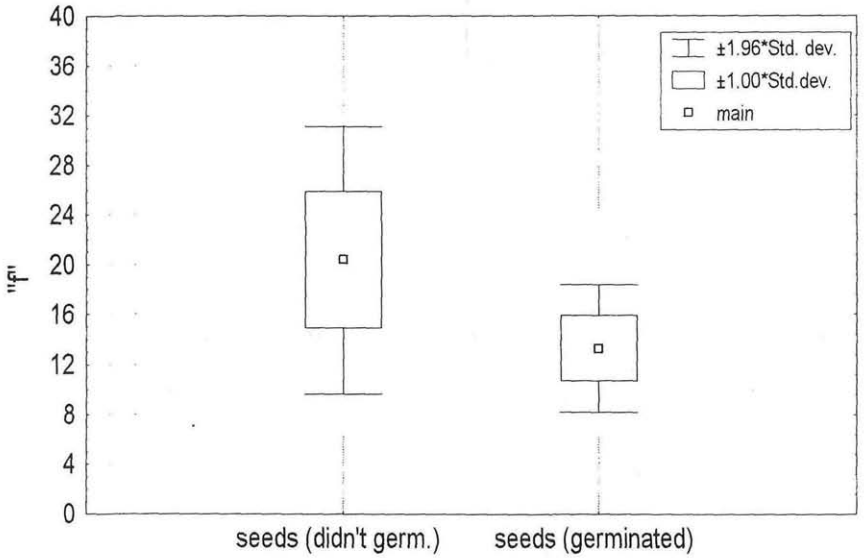

Fig. 5: Vitality coefficient for common maple seeds

Table 3:

Vitality coefficient »f« and germination test of common maple seeds

series	I		II		III		IV		V	
test N°	f	g	f	g	f	g	f	g	f	g
1	16	-	21	-	20	-	13	-	13	+
2	18	-	20	-	14	+	21	-	12	+
3	21	-	12	+	11	+	14	+	12	+
4	30	-	12	+	11	+	13	+	18	+
5	16	+	13	+	14	+	21	-	15	+
6	11	+	25	-	15	-	16	+	13	+
7	14	+	12	+	19	-	18	-	18	-
8	13	+	14	+	16	-	21	-	11	+
9	23	-	14	+	14	+	12	+	13	+
10	23	-	15	+	18	-	22	-	18	+
11	15	+	11	-	15	+	19	-	13	+
12	11	+	15	-	12	+	22	-	23	-
13	12	+	12	+	13	+	14	+	14	+
14	15	+	8	+	15	+	10	+	15	+
15	14	+	15	+	15	+	13	+	15	+
16	12	+	14	+	11	+	7	+	15	+
17	13	+	13	+	14	+	10	+	17	+
18	13	+	14	+	18	-	19	-	16	+
19	9	+	14	+	17	-	15	-	14	+
20	13	+	20	-	14	-	19	-	19	-

series	VI		VII		VIII		IX		X	
test N°	f	g	f	g	f	g	f	g	f	g
1	14	+	20	-	21	-	15	+	20	-
2	20	-	15	+	14	+	14	+	12	+
3	30	-	22	-	16	+	10	+	8	+
4	16	+	32	-	11	+	19	-	17	+
5	12	+	15	+	23	+	15	+	11	+
6	18	-	10	+	9	+	9	+	9	+
7	16	+	16	+	11	+	24	-	14	+
8	12	+	13	+	7	+	19	+	11	+
9	15	+	35	-	10	+	22	+	19	-
10	11	+	27	-	12	+	14	+	15	+
11	13	+	20	+	13	+	21	-	23	-
12	11	+	22	-	12	+	13	+	12	+
13	16	+	18	+	14	+	16	+	14	+
14	22	-	16	-	15	+	13	+	18	-
15	18	+	14	+	10	+	12	+	16	-
16	23	-	14	+	14	+	17	-	21	-
17	15	+	12	+	8	+	16	+	15	+
18	19	-	13	+	11	+	15	+	16	-
19	14	+	10	+	38	-	17	+	16	+
20	17	-	17	-	42	-	11	+	12	+

Legend: **f...** vitality coefficient
g... germination text: +... seed which geminated
–... seed didn't germinate

Table 4

Pearson contingency coefficient (cc) and X^2 significance test

	common maple				
series	I.	II.	III.	IV.	V.
X^2	11,7***	11,7***	1,6	5,5*	6,0*
cc	0,61	0,61	0,27	0,46	0,48

	common maple				
series	VI.	VII.	VIII.	IX.	X.
X^2	9,3**	12,9***	14,1***	4,8*	6,6*
cc	0,53	0,63	0,64	0,44	0,50

Significance: 5%... *
1%... **
0,1%... ***

Conclusion

The experiment proved that the high frequency electrophotograpic method gives results in agreement with the biochemical method using tetrazolium (TTC). The high frequency method showed certain advantages:

- method could be repeated on the same seeds, which gives the opportunity to monitor any change of seed vitality with time;
- seeds are intact and living after passing the test – thus comparative tests could be performed with some other methods;
- the high frequency method is faster and cheaper, and the test does not require the use of expensive chemicals;
- additional treatment of the seeds is not necessary (such as soaking and warming before the test);
- the photographs are permanent and the results are immediate.

In spite of agreement with classical tests, the variation of vitality coefficient values is too wide and at this point we are unable to separate the factors which are causing that variety. Therefore the method could be used only for comparative purposes and not as an independent one.

Technically it is possible to observe and take photographs of only one seed at a time. The seed must be alligned on one of its flat areas, so not all seeds from all species could be tested.

The frequency of the device was also lower (1400 s^{-1}–3000 s^{-1}) than the frequency cited in the literature for observation of living creatures (30 000 s^{-1}) (JOHNSON 1977).

All of mentioned insufficiencies could be technically solved, so the efficiency and simplicity of the test would be increased; for example a Polaroid film would eliminate all the work in the darkroom, etc.

This study attempted to show the possibility of using alternative, until recently non conventional methods, which might provide a different approach and solution to the problem.

The use of high frequency discharge photography in the natural sciences is at its very beginning and determination of seeds vitality could be only one of many other approaches to simplify recent methods and research.

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Phyton (Horn, Austria) 38 (2): 237–238 (1998)

Recensio

ENDRESS Peter K. 1996. Diversity and Evolutionary Biology of Tropical Flowers. – Cambridge Tropical Biology Series. – Gr. 8°, XIV + 511 Seiten, zahlreiche Abbildungen (schwarz-weiß); Paperback. – Cambridge University Press. – £ 24,95. – ISBN 0-521-56510-3.

Dieses zuerst 1994 in einer gebundenen Ausgabe erschienene Werk liegt nun in einer korrigierten Paperback-Version vor. In der Einleitung schildert der Autor die Entdeckungsgeschichte und den fragmentarischen Kenntnisstand von *Delonix regia*, dieser allgemein kultivierten, aber am natürlichen Standort in Madagaskar vom Aussterben bedrohten Art als ein Beispiel für den erstaunlich geringen Erforschungsgrad auch auffälliger, bekannter tropischer Arten (zu dem er für die ge-