On the Cause of 'Hardness' in the Seeds of *Indigofera arrecta*.

BY

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With Plate VII.

IT is well known that seeds possessed of hard coats, which do not allow of the penetration of water and consequent germination, frequently occur in certain leguminous plants, but no satisfactory explanation of the cause of this phenomenon has, so far as we are aware, been yet given. Attention has been drawn to the very marked case of hardness in the seeds of *Indigofera arrecta* by Leake (1), who points out that in the seed of this plant derived from Natal or grown in India the germination capacity normally varies from 5 to 10 per cent., but that by suitably scratching the seed-coat, in the manner commonly practised in the case of clover, it may be made to very nearly approach 100 per cent. These observations have been confirmed, and have been found to apply equally to seed derived from Java. The matter has assumed considerable industrial importance owing to the introduction of *Indigofera arrecta*, as a substitute for the previously grown *Indigofera sumatrana*, into the indigo districts of Bihar, and machines have been devised for scarifying the seed in bulk, in such a manner as to render its germination capacity satisfactory.

It has recently been found that the same end could be gained more simply, and with more certainty of success, by treating the seed with concentrated sulphuric acid; the seed resists the attack of this substance for twenty minutes to half an hour, and, after washing and drying, is found to germinate perfectly¹.

As a result of this observation it seemed to us of interest to try to determine to what cause the hardness of the seed was due, and how this cause was removed by scarification, and by treatment with acid. The only suggestion we have been able to trace as to the cause of hardness in seeds

¹ The idea of using sulphuric acid arose out of certain experiments carried out by Dr. Butler on the destruction of the eggs of insect pests in the lint of cotton-seed by treatment with this substance, after which, the germination capacity of the seed was found to be considerably improved.

is made by Percival (2) to the effect that their testae contain an abnormally large amount of ash constituents, and that these resist the penetration of water. This explanation has been shown inadequate in the case under consideration by Leake (loc. cit.), who compared the amount of ash derived from testae of seeds of *Indigofera arrecta* with that obtained from the seed of *Indigofera sumatrana* (with the germination of which there is no difficulty) grown in the same locality, and found a considerably larger quantity in the latter case.

We have therefore sought for an explanation in the structure, or the nature, of the organic constituents of the seed-coat.

Preliminary examination of sections of the testa in an unstained condition, or treated with a simple undifferentiating stain (e. g. carmine or haematoxylin), showed its structure to be identical with that characteristic of the seeds of other leguminous plants. Superficially there is a layer of thick-walled 'palisade' cells with their longer axes parallel to the radii of the seed; beneath this is another layer of thick-walled cells of peculiar shape whose contents are pigmented yellow, and beneath this layer again, a double row of elongated cells with their longer axes parallel to the circumference of the seed; finally, there are several layers of ordinary parenchymatous tissues (Pl. VII, Fig. 1). No essential difference could be found in the structure of the testae of the seeds of *Indigofera arrecta* and *Indigofera sumatrana*, and in neither case could any traces of substances of a waxy nature be detected, either by examination of untreated sections or sections treated with osmic acid.

In order to ascertain how far water could penetrate into the hard seed-coat, sections were cut of seeds which had remained in water containing a little fuchsin for twelve hours without showing any signs of swelling. It was found that the stain had not penetrated beyond the outermost layer of the seed, and was deposited there in a sharp line (Fig. 1). On similarly examining seeds of *Indigofera sumatrana*, or those of *Indigofera arrecta* which had been scarified or previously treated with sulphuric acid, the stain was found to penetrate to the innermost layer of the testa (Fig. 2).

This seemed to clearly indicate that the resistant layer was to be found at the extreme outside of the hard seeds, and a careful examination was therefore made of the chemical nature of the substances composing the 'palisade' cells. On treating sections of such seeds with chlor-zinc-iodine (Schulze's solution), the cell-walls of all the tissues swelled and acquired the violet colour characteristic of cellulose, and the cell-contents the yellow colour indicating the presence of proteid substances. No cuticle could be detected by this treatment, and sections of the testa of *Indigofera sumatrana* seeds, and those of *Indigofera arrecta*, after treatment by scarifying or acid, presented an identical appearance to those of untreated

hard seeds. No explanation of the resistance to water in the latter case seemed, therefore, to be provided by this method. Sections treated with sulphuric acid and iodine lead to precisely similar results (Fig. 3).

Several attempts were made to demonstrate the existence of a cuticle in sections of hard seeds by methods of double staining (e. g. aniline-safranin counterstained with haematoxylin, and Gram-eosin), but in no case could a layer reacting towards these substances in the manner typical of cuticle be shown.

An explanation was eventually found by treating sections with a delicate stain for differentiating cellulose and cuticle described by Mangin (Zimmermann, 3), consisting of a solution of iodine in phosphoric acid. On treating sections of the testa of *Indigofera arrecta* seeds with this reagent, the cell-walls of all the tissues and the cell-contents reacted as cellulose and proteid matter respectively as before, but after a few minutes' action, a thin yellow line was seen to appear at the extreme periphery of the section, and to gradually swell as time progressed. On first appearing, this cuticular layer was only about $3-4\ \mu$ in thickness, but in the course of a quarter of an hour it had become more than three times this depth and was very clearly discernible (Fig. 4). Eventually the epidermal cells swelled enormously and ruptured, whilst the cuticle split away and was seen as a broad yellow band (Fig. 5). On treating sections of seeds of *Indigofera sumatrana* or of 'treated' *Indigofera arrecta* with this reagent no layer of this kind could be discerned. It was subsequently found that the cuticular layer characteristic of the seeds of *Indigofera arrecta* could be shown in sections treated with a concentrated alcoholic solution of chlorophyll, as recommended by Correns (4), but the test is difficult of application and uncertain in result.

It is clear, therefore, that the hard nature of the seeds of *Indigofera arrecta* is due to their possession of a very thin outer covering of a substance resistant to water. The exact nature of this substance cannot be determined by the methods which have been described. By its reaction under treatment with chlor-zinc-iodine, its swelling under the action of dehydrating agents, and its failure to respond to double staining methods, it would appear to consist of cellulose; whilst by its colour reaction towards Mangin's phosphoric acid and iodine, its staining with an alcoholic solution of chlorophyll, and its impermeability to water, it would seem to be cuticle. We can only conclude that it consists of a body of an intermediary nature, possibly a transition-product, between cellulose and cuticle.

The action of scarifying the seed is, no doubt, to remove a portion of this resistant covering, and so allow of the penetration of water, whilst sulphuric acid acts either in a similar manner by leading to the swelling of this layer and its eventual rupture, or by converting it into a body akin to cellulose and permeable to water.

It would be of interest to determine whether this latter method of treatment would answer in other cases of hard seed, and whether their hardness can be traced to a similar cause to that which has been described.

LITERATURE.

1. H. M. LEAKE : Journ. Royal Hort. Soc., vol. xxix.
 2. J. PERCIVAL : Agricultural Botany, p. 623.
 3. A. ZIMMERMANN : Die botanische Mikrotechnik, p. 138.
 4. ————— : Ibid., p. 149.
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Since writing the above we have seen a paper by Jarzymowski (Inaugural-Dissertation, Halle, 1905), dealing with hardness in several leguminous seeds. This author holds that hardness is conditioned by the size of the lumina in the cells of the ‘palisade’ layer of the seed-coat. This explanation does not apply to the case of *Indigofera arrecta*. No difference can be detected between the shape or size of the lumina in such cells in the coat of this seed and in that of *Indigofera sumatrana*, nevertheless the former hardly germinates at all without treatment, and the latter germinates freely. The presence of the hard layer which has been described in the hard variety and its absence in the variety in which hard seeds do not occur, seems to point clearly to this being the determining cause in this case.

Jarzymowski has tested the method of treating hard seeds with sulphuric acid on several varieties and finds it to answer well ; it appears to have been first suggested by Hiltner in 1902.

Bergthil, C. and Day, D. L. 1907. "On the cause of 'hardness' in the seeds of *Indigofera arrecta*." *Annals of botany* 21, 57–60.

<https://doi.org/10.1093/oxfordjournals.aob.a089123>.

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