Observations on the Parasitic Behaviour of
Thesium humile Vahl. (Santalaceae)

II. Thesium Fruit and its Uptake of Water

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ABSTRACT

Thesium fruit (kernel) is small, about 2.0 × 3.5 mm which is surrounded by a pericarp of thin dissoluble outer layers and hard thick inner one. The dissolution of the outer layers, besides being a rich cultural medium for fungal growth, forms a thick mucilagenous mass which is responsible for the higher matric potential of *Thesium* kernels.

Matric potential of *Thesium* kernels was estimated by interpolation of the osmotic potentials of the bathing solutions, and by calculation from the vapour pressure measurements using Raoult's law. The reliability of the first method, which gave a value of about −1,000 bars, is discussed. Using vapour equilibration method, a value of −708 bars, which is about twice that of *Hordeum* grains, was recorded. The still high matric potential of *Thesium* kernels is considered as an adaptation to the parasitic behaviour of this species.

The change in weight of the slit and the intact kernels when alternately subjected to two different relative humidities indicates a slight impermeability of kernel coat. There is, however, no indication that the hilar fissure of *Thesium* kernels exerts a control on water entering it.

INTRODUCTION

Earlier work on the hemiparasite *Thesium humile* var. maritima Simps. reported by Abou-Raya *et al.* (1), has shown that it is of common occurrence in Libya in both the grassland and the arable crops. It infects the roots of a wide variety of host plants, especially the barley plant *Hordeum vulgare* L. It should be noted at the outset that this hemiparasite represents a serious danger to that crop in some districts.

This paper covers the anatomy of *Thesium* fruit, its uptake of water during inhibition, and evaluation of its matric potential.

MATERIAL AND METHODS

Fruits of *Thesium humile* var. maritima Simps. were collected in the Spring of 1977 from one of the heavily infected barley fields located in Jefara Plain about 10 km south

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of Tripoli International Airport. The collected fruits from the fields were cleaned by separating the foreign matter and other tissues of plant, and then were kept in air-tight glass jars and stored in the laboratory for fruit testing.

The standard microtome technique, wax embedding and staining was followed for sectioning the kernels. Selected sections of the kernel were carefully observed under the light microscope fitted with a camera lucida. Hand drawings were made to reveal the internal anatomical structure of the sectioned kernels.

To determine the capacity of kernels to imbibe water, about 2 cm of air-dry kernels were soaked in distilled water in a petri dish and weighed periodically after drying the outer surfaces with filter papers. The increase in weight was recorded as percentage of air-dry weight of kernels.

For estimation of imbibition pressure of kernels, the method used by Shull(11) with *Xanthium* seeds was followed. Sets of air-dry kernels, 2 gm each, were soaked in solutions of NaCl and LiCl of various osmotic potentials. Changes in weight after 48 hours of soaking were recorded as percentage of air-dry weight of kernels.

To obtain a more satisfactory value of the matric potential of the kernels, a modified method of Slatyer (8) was introduced in the present investigation. Since all the NaCl and LiCl solutions used have less affinity for water than the imbibant, it was thought that a realistic matric potential value of kernels might be approached by subjecting the imbibant to a dry atmosphere so that the moisture in the kernels may forcibly diffuse out in form of water vapour, Sulphuric acid was used for this purpose and this enabled us to evaluate the matric potential of *Thesium* kernels in terms of relative humidity. Series of sulphuric acid solutions of different concentrations, in addition to solutions of NaCl, provided a wide range of relative humidities. Fixed amounts from these solutions were introduced into test tubes of 12 cm × 1.5 cm each. Small bags made of muslin fabrics were filled with about one gm of air-dry kernels, and were suspended in the closed spaces from the lower end of the tightly fitted rubber stoppers above these solutions. The test tubes were then kept in an incubator maintained at 20°C. Using a torsion balance of one mg sensitivity, the percentage change in the weight of air-dry kernels after attaining equilibrium, was recorded. The air-dry grains of barley, *Hordeum vulgare* L., were tested in the same way for the sake of comparison with *Thesium* kernels.

By knowing the absolute temperature (T), the vapour pressure of pure water at that temperature (P^0), the vapour pressure in the enclosed space (P), and the partial molar volume of water (V_1 in litre/mole), the water potential is calculated using the following formula as derived from Raoult's law:

$$-\psi = \frac{RT}{V_1} \, Ln \, \frac{P^0}{P}$$

As stated by Salisbury and Ross (10), the above law is simplified to:

$$-\psi = 10.7 \, \log_{10} \left(\frac{100}{R\,H} \right).$$

By applying this formula, the water potentials of the solutions used could be calculated if we know their relative densities as in the case of sulphuric acid solutions (13), or their molarities as in the case of NaCl solutions and their corresponding relative humidities.

RESULTS AND DISCUSSION

1. Morphology and Anatomy of Thesium Fruits:

Thesium fruits, an average of 20–30 per plant, develop singly in the axils of slender lanceolate leaves. Each fruit is small, globose, inferior nut (kernel), crowned by a persistant perianth (12). The eval-shaped green or yellowish green kernels are relatively small, 2×3.5 mm in average size, and possess 2 to 7 prominant ridgres which run parallel to the long axis. The rest of the pericarp wall is reticulated (Fig. 1a). The main reticulation is due to the thickening of the lateral and internal walls of the endocarp cells. These walls are highly lignified. Radial longitudinal and transverse sections (Fig. 1b,c) showed that the pericarp occupies about 50% of the kernel volume.

The pericarp itself consists of three distinct layers; the thin dissoluble outer two layers which extend to about the internal part of the hilar fissure, and eventually come in contact with the embryo, and the thick inner one. When *Thesium* kernels were soaked in water, the dissolution of out layers forms a thick mucilagenous mass which, besides being responsible for the high matric potential, it forms a rich cultural medium for fungal growth. The importance of this high martic potential was discussed below, but the significance of this fungal growth in the germination process of this species will be discussed in an another paper of this series. Inside the pericarp there is another distinct and thin layer which is probably the testa (2).

The differentiated embryo, which has two small cotyledons, occupies the central part and has large accumulation of fats in the endosperm which occupies more than 50% of the kernel volume (Fig. 1b,c). Eckery and Miller (5) had also reported the presence of fats and oils in some genera of the family Santalaceae.
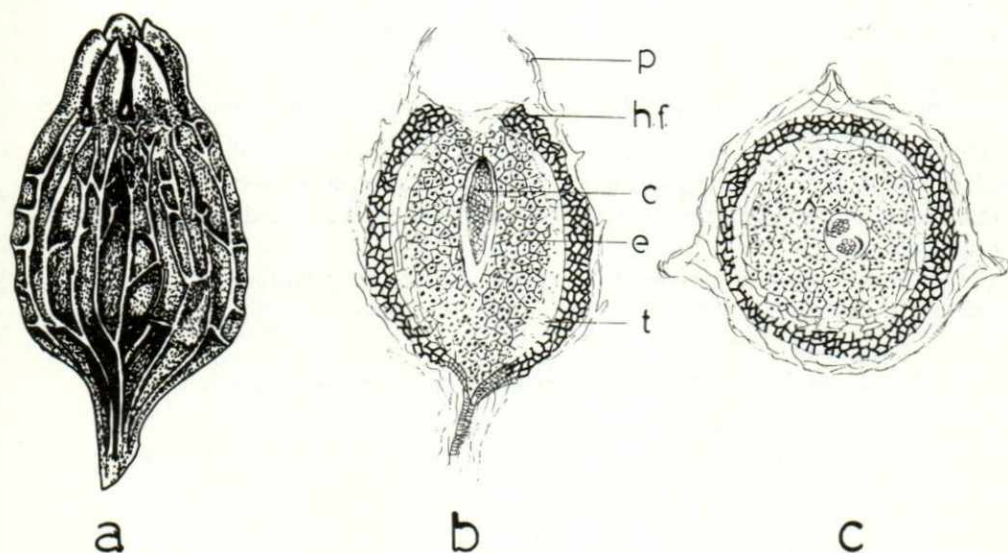

Fig. 1.   Morphological and anatomical details of *Thesium* kernels; a) entire kernel × 20, b) radial longitudinal section × 20, c) transverse section × 20, showing; P) persistant perianth; h.f.) hilar fissure; c) cotyledons; e) endosperm; t) testa.

II. Imbibition of thesium kernels soaked in pure water:

The amounts of water imbibed as percentage of air-dry weight of *Thesium* kernels, after being soaked is distilled water for given periods, are represented graphically in Fig. 2. The rapid increase in kernels weight at the beginning of the soaking period was quite noticeable. In six hours the kernels imbibed an amount of water equal to its own weight. After 48 hours of soaking, the weight of kernels was almost 3 times that of their initial dry weight. After this period the dissolution of the outer kernels coat makes the extent of the readings almost impracticable. Moreover, the mucilagenous diffusate from the soaked kernels could be noticed after few hours of soaking. What is more important was the growth of the heavy web of fungal mycelium covering almost all the kernels as if that diffusate is a cultural medium.

III. Imbibition of water from solution of different osmotic potentials:

The solutions used in the experiment with their corresponding osmotic potentials as reported by Shull (11), and the amount of water imbibed after 48 hours of soaking are presented in Table. 1. It is interesting to note that even saturated LiCl would not prevent hydration of the soaked kernels. Thus it is tempting to suggest that the matric potential of *Thesium* kernels, based on the solution method, might be more than $-1,000$ bars, a value even higher than that recorded by Shull (11) for *Xanthium* seeds. In attempting to evaluate the imbibition pressure of *Xanthium* seeds, Shull (11) immersed these seeds in solutions of different osmotic potentials. Since almost all of these solutions have lower affinity for water than the imbibant, and since the relation between the osmotic potential and the amount of water imbibed is not a strictly proportional one, the straightforward interpolation of the above data for the evaluation of matric potential is not exactly correct. Therefore, it was deemed important to estimate the matric potential using a modified technique (8) for the measurements of vapour pressure so that the above mentioned drawbacks, as evident in Shull's method, might be eliminated.

IV. Evaluation of matric potential of thesium kernels in terms of relative humidities:

The NaCl solutions and sulphuric acid concentrations with their corresponding relative humidities, and the percentage change in the weight of air-dry *Thesium* kernels and barley grains after attaining equilibrium, are presented in Table 2 and illustrated graphically in Fig. 3.

In order to ascertain a computed value of the matric potential of the kernels, the data of the percentage change in dry weight (Y) as a function of the water potential of

Table 1   Imbibition of thesium kernels as affected by different osmotic pressure.

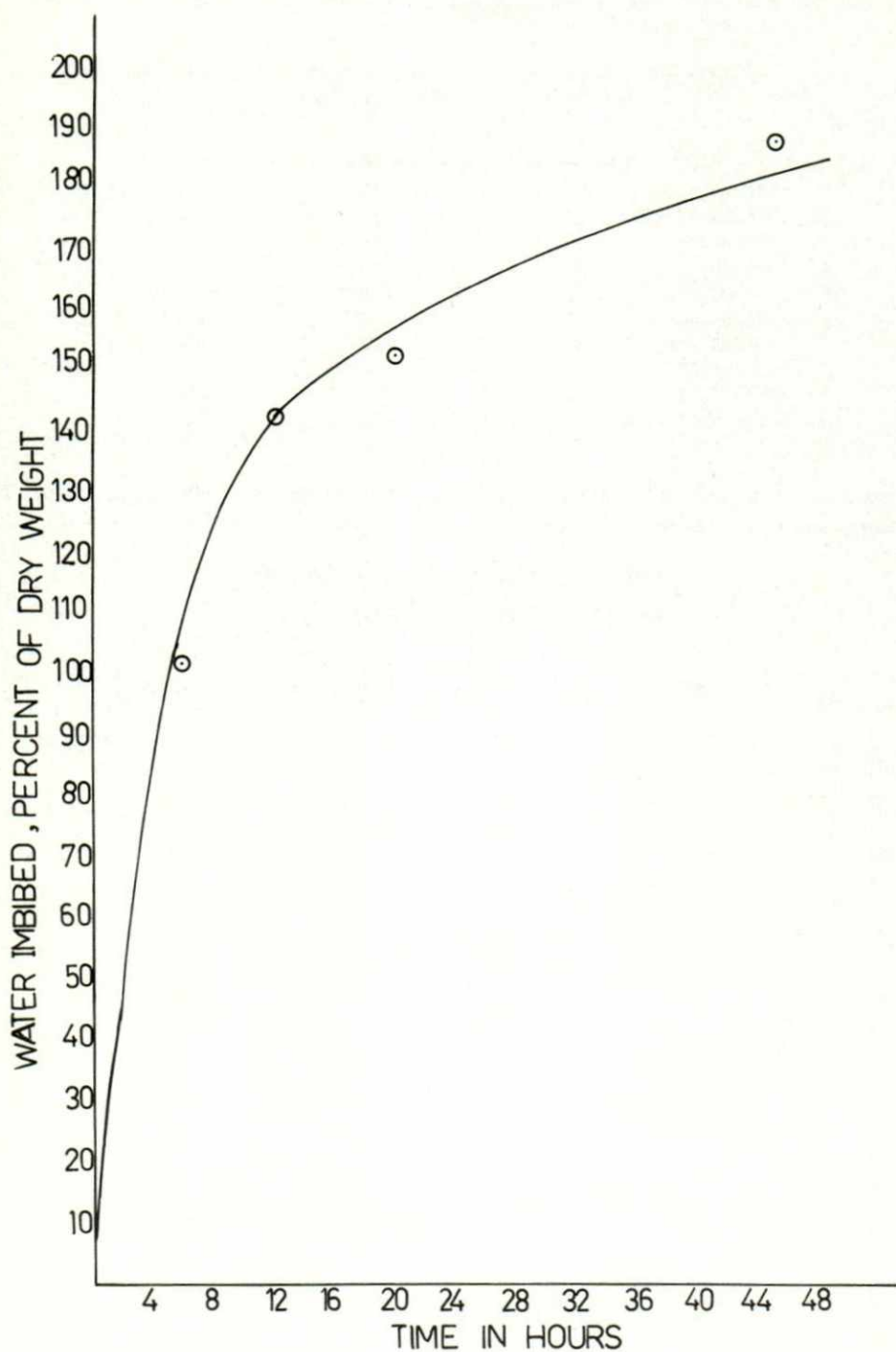| Volume molar concentration of solution	Osmotic potential of solutions, bars.	Water imbibed by seeds at equilibriums (48 hours). Per cent of air dry weight.
2 M NaCl	$-$ 72.9	176.1
3 M NaCl	-105.4	156.8
4 M NaCl	-131.7	137.1
Sat. NaCl	-379.9	121.3
Sat. LiCl	-977.7	26.3

Fig. 2. Percentage change in weight of air-dry *Thesium* kernels soaked in distilled water for given periods.

Table 2 Percentage change in weight of air-dry Thesium kernels and Hordeum grains in atmospheres of different relative humidities.

No.	Solutions	Relative humidity (%)	Osmotic potential of solutions, bars	% change in weight of air-dry fruits	
				Thesium	Barley
1	H_2SO_4	10	−2930	−5.90	−7.28
2	H_2SO_4	20	−2051	5.40	−7.22
3	H_2SO_4	30	−1630.3	−4.57	−6.68
4	H_2SO_4	40	−1254.0	−3.87	−6.40
5	H_2SO_4	50	−940.5	2.67	−5.80
6	H_2SO_4	60	−689.7	−0.99	−4.75
7	H_2SO_4	70	−470.3	−0.22	−4.54
8	5.0 M NaCl	78.4	−344.9	6.48	1.70
9	4.0 M NaCl	83.0	−250.81	13.50	2.63
10	3.0 M NaCl	88.3	−156.76	18.45	5.59

the used solutions (X) were transformed to fit a linear regression relationship. Thus, the Y-values were first transformed into:

$$\text{Log } (Y + 5.91) = Z \quad \text{for } Thesium, \text{ and}$$
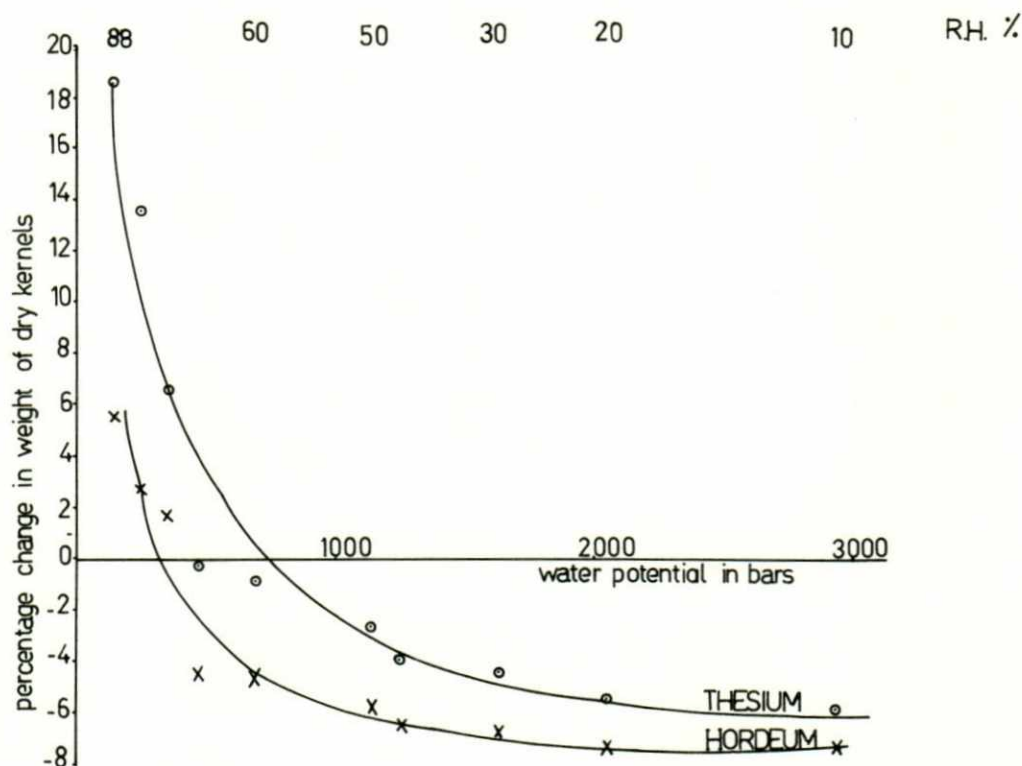$$\text{Log } (Y + 7.29) = Z' \text{ for barley,}$$

Fig. 3. Percentage change in weight of air-dry *Thesium* kernels subjected to different water potentials as computed from their corresponding relative humidities.

where $Y = \%$ change in weight of kernels and grains,

Z = transformed values for *Thesium*,

Z' = transformed values for barley,

and 5.91 and 7.29 are the two figures which just exceed those of the minimal Y-values attained by *Thesium* and barley respectively. This was done to ensure that all the Y-values are defined.

Then, a linear regression of Z-values and Z'-values on X-values was fitted. With respect to *Thesium*, this gives:

$$Z = 3.5020 - 0.002436 \ X,$$

and for $Y = 0$,

$$Z = \text{Log } 5.91 = 3.5020 - 0.002436 \ X$$

Therefore $X = -708.3$ bars.

For barley, this gives:

$$Z' = 2.9299 - 0.002499 \ X,$$

and for $Y = 0$,

$$Z' = \text{Log } 7.29 = 2.9299 - 0.002499 \ X$$

Therefore $X = -377.5$ bars.

From these computations it appears that the estimated matric potential of *Thesium* kernels is about twice the negative value of that of barley grains. This value is still a surprisingly high one. However, in the authors' view, the estimation of the matric potential from the vapour pressure measurements not only overcomes the drawbacks in Shull's method but also has some advantages. Besides allowing to use a wide range of relative humidities, it saves the complications resulting from immersing the imbibants in liquid solutions. It is noteworthy, moreover, that in case of *Thesium* kernels in which dissolution of their outer layers occurs, the only reliable and most suitable method is the vapour equilibration method applied in this study.

V. Control of water imbibition by thesium kernels:

It was thought that hilar fissure of *Thesium* kernels might exert a control on water entering the kernel. This was tested by measuring the changes in moisture content of slit (cracked) and intact kernels after they have been alternately subjected to two different relative humidities. The same technique used for the estimation of matric potential through measurements of vapour pressure was followed in this set of experiments. Distilled water and sulphuric acid concentration were used to attain 100% and 10% relative humidities in the enclosed space of test vials.

Data of percentage change in weight of air-dry kernels as a function of time as they were subjected alternately to 100% and 10% R.H. are presented in Table 3 and illustrated in Fig. 4. It is apparent that, irrespective of the order through which the kernels were alternately subjected to the different relative humidities, moisture changes of the slit and the intact kernels paralleled each other. They both rise or fall relative to humidity treatments indicating no control of that orifice to the entering of water vapour into the *Thesium* kernels. However, the rate of water gain or less showed an apparent difference between the slit and the intact kernels. At 20 to 30 hours after treatment, the amount of water absorbed or lost by the slit kernels was about 100%

Table 3   Percentage change in weight of air-dry kernels at 100% and 10% relative humidity

Time (hours)	A*				B*			
	100 RH		10% RH		10% RH		100% RH	
	intact	cracked	intact	cracked	intact	cracked	intact	cracked
20	7.3	15.8			−4.7	−9.8		
46	14.6	23.9			−3.8	−7.3		
	25.8	30.4			−4.7	−8.3		
132	24.4	31.2			−3.9	−8.2		
160			−8.3	−10.0			14.0	20.5
187			−9.6	−10.5			25.7	28.9
211			−10.1	−10.8			30.3	33.9
237			−10.2	−10.9			33.9	35.2
262	4.6	5.5			−1.0	−1.6		
311	14.7	17.8			−1.6	−2.2		
359	18.5	19.7			−1.8	−2.3		

*'A' Kernels subjected to 100% R.H./10% R.H./100% R.H.
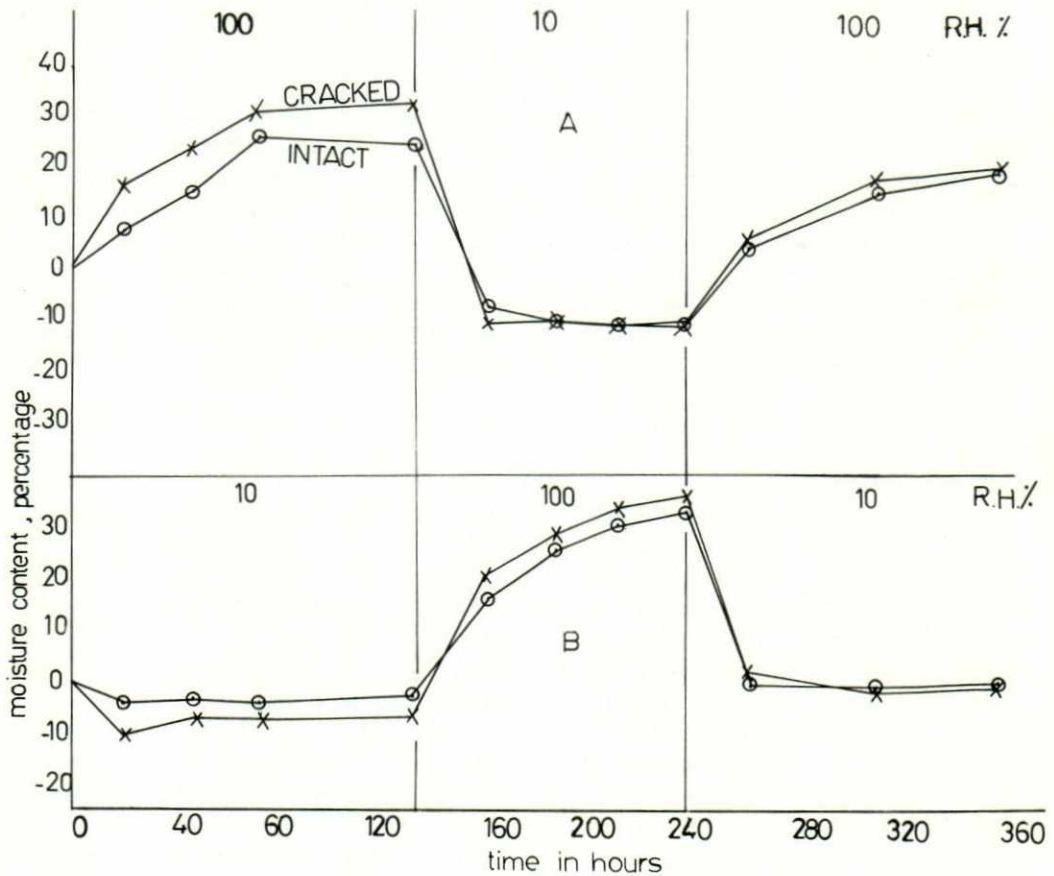*'B' Kernels subjected to 10% R.H./100% R.H./10% R.H.

Fig. 4.   Percentage change in weight of slit and intact *Thesium* kernels when they are alternately subjected to 100% R.H. and 10% R.H.

greater than that of the intact kernels. However, this difference in water uptake or loss was greatly reduced as the period of treatment increased. These results might indicate that the coat of *Thesium* kernels is slightly permeable to water vapour.

In some legume seeds, in which the hilar fissure develops a highly specialized hygroscopic tissue, Hyde (6), and Devlin (4) described a mechanism by which this hilar fissure controls the water entering them. In such seeds the moisture content never rises when they are transferred from low to a high relative humidity, and always falls when they are transferred from high to low relative humidities. In *Thesium* kernels, the moisture content of both the slit and the intact kernels rises and falls relative to humidity treatment indicating the absence of such mechanism. This might be attribute to the absence of hygroscopic tissue in the hilar fissure (Fig. 1b).

Thesium sp., like the other hemiparasites does not require root exudate from the host to initiate germination and to ensure subsequent contact with it. Moreover, this Santalean species, unlike some of the hemiparasitic Scrophulariaceae studied by Chancellor (3), and Klaren (7) is capable of autotrophic existence only in the very early stage of its life cycle.

From the present studies and currently available data on germination (unpublished), it appears that *Thesium* kernels initiate its germination aided by an imbibitional force which is so high that its uptake of water could be accomplished in a relatively dry atmosphere. It is of a special interest, from an agronomic point of view, to note that the short-lived independent stage of *Thesium* growth is characterised by a very well developed root system which ensures self support and contact with the host's roots at an early stage; of well developed chlorophyll pigments in the seedling leaves make the species capable of synthesizing organic food through photosynthesis (unpublished). This short-lived independent stage of *Thesium* growth, which is crucial to its existence, can sustain unfavourable environmental conditions aided by these extraordinary characteristics.

ACKNOWLEDGEMENT

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مشاهدات على المسلك التطفلي لنبات « مقلية الراعي » (من الفصيلة الصندلية)

٢ — ثمارنبات « مقلية الراعي » وتشربها للماء

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المستخلص

يتطفل نبات « مقلية الراعي » على جذور بعض النباتات ، خصوصا نبات الشعير . ويحمل هذا النبات ثمارا صغيرة لا يتجاوز حجمها ٣٫٥×٢ مم ، وتحاط بجدار من ثلاث طبقات : الاثنتان الخارجيتان منها سرعان ما تتحلل أثناء نقعها في الماء ، أما الطبقة الثالثة الداخلية فسميكة . ونتيجة لتحلل هاتين الطبقتين يتكون وسط غذائي تنمو عليه ميسيليوم الفطريات بوفرة ، كما وأن تحلل هاتين الطبقتين قد يكون هو السبب في ارتفاع ضغط التشرب لهذه الثمار .

وأمكن تقدير ضغط تشربها بطريقتين : إما بتقدير الضغط الازموزى للمحلول الذى يتزن مع هذه الثمار عند نقعها فيه ، أو بتطبيق قانون « رآولت » وحساب الضغط البخاري الذى فوق المحلول والذى يتزن معها .

والقيمة التي أمكن الحصول عليها من الطريقة الأولى هي (– ١٠٠٠) بار ، وقد نوقش مدى الاعتماد عليها . ومع أن القيمة بالطريقة الثانية لا تتجاوز (– ٧٠٨) بار ، إلا أنها مازالت عالية ، حيث تبلغ ضعف ضغط التشرب لحبوب الشعير إذا قورنت بها .

وواضح أن ارتفاع ضغط التشرب لثمار مقلية الراعي يتوافق تماما مع المسلك التطفلي لهذا النبات .

ويختلف وزن الثمار الصحيحة لهذا النبات ، اختلافا طفيفا ، عن وزن ثماره التي تم كسرها إذا ما تعرض كل منها لدرجتين مختلفتين من درجات الرطوبة النسبية بالتناوب ، ويشير ذلك إلى قلة نفاذية غطاء الثمرة لبخار الماء ، ومع ذلك فلا يوجد ما يدل على تحكم ثقب النقير في حركة بخار الماء من وإلى داخل الثمرة .