# Notes.

is quite distinct from R. antennulifera. But this second species rather weakens than strengthens *Eminia* as a genus, and it is proposed to treat it as a section of *Rhynchosia* here.

*Rhynchosia* (§ *Eminia*) *Holubii*, Hemsl. nov. spec.: foliis per anthesin persistentibus, foliolis oblongis sinuatis; racemis folia vix superantibus; calyce quam corolla dimidio breviore, laciniis 3 inferioribus saepius 2-3 glandulosis; stylo per totam longitudinem glabro.

Herba, ut videtur, perennis, caulibus . . . ? Folia graciliter petiolata, pinnatim trifoliolata, coriacea, strigillosa, cum petiolo 3-5 poll. longa; foliola breviter petiolulata, oblonga vel anguste ovatooblonga, 2-3 poll. longa, lateralia paullo minora, sinuata vel obscure paucicrenata, utrinque obtusissima, simul apice minute apiculata, supra parce strigillosa, scabrida, venis elevatis grosse reticulatis, subtus argentea, dense strigillosa, venis primariis lateralibus utrinque circiter 5, stipellis obsoletis. Flores circiter 9 lineas longi, fasciculatoracemosi, brevissime pedicellati, racemis axillaribus folia subaequantibus calyceque argenteis strigilloso-hirsutis; calyx sub-bilabiatus, alte inequaliter 4-fidus (i. e. laciniis 2 superioribus fere ad apicem connatis), lacinia inferiore saepius apice 3-glandulosa, laciniis lateralibus saepius 2-glandulosis, labio superiore saepius eglanduloso; petala fere aequilonga, unguiculata, glabra, vexillo lato leviter emarginato; stamina glabra; ovarium subsessile, apice praesertim longissime setulosum, stylo longissimo breviter exserto glabro. Legumen ignotum.

Apart from the great difference in foliage, and apparently in habit, this species differs in the relative length of corolla and calyx, and especially in the two upper calyx-lobes being connate nearly to the apex and eglandular, and in the three lower being furnished with two or three glands at the apex.

### W. BOTTING HEMSLEY, Kew.

**ON THE PRESENCE OF A DIASTATIC FERMENT IN GREEN LEAVES.**—I have been led to re-investigate this subject in consequence of the statement made by Wortmann<sup>1</sup> in a paper recently published, that green leaves contain no diastase or only such a minute quantity that it cannot be of any serious importance in the transformation of starch into sugar which takes place so actively in those organs. In contradiction of the results obtained by Baranetzky<sup>2</sup>

- <sup>1</sup> Botanische Zeitung, 1890.
- <sup>2</sup> Die stärkeumbildenden Fermente in den Pflanzen : Leipzig, 1878.

and of Brasse<sup>1</sup>, which seem to prove the presence of diastase, Wortmann finds that watery extracts of green leaves have no diastatic action, and concludes that the normal conversion of starch into sugar is effected in the living leaf by the direct action of the protoplasm.

I am unable, within the limits of a note, to criticise Wortmann's experiments in detail, or to give a full account of my own. In the way of criticism I would only point out that Wortmann's method is open to serious objection: the mixing of a certain quantity of leafextract with a certain quantity of starch-solution, and then using the iodine-reaction as a means of determining the amount of chemical change effected, is not a method calculated to give definite results; for, as Wortmann himself admits, the colouring-matters and other foreign substances in the leaf-extract interfere with the colour-reactions with iodine. Again, it is disadvantageous to filter, as Wortmann did, the watery extract of the leaves before adding it to the starch-solution. I have found that the turbid extract, merely strained, is much more active than the clear extract obtained by filtration : probably Wortmann's negative results were mainly due to this cause.

There is one experiment upon which Wortmann lays so much stress that it calls for special notice. He finds that the starch will not disappear from the cells of a living leaf if the leaf be kept in an atmosphere of carbon dioxide; and from this he concludes that the transformation of starch into sugar must be a direct vital act of the protoplasm, dependent upon the supply of free oxygen. Assuming the correctness of the observation, a more simple and satisfactory explanation of it would be that, in the absence of free oxygen, the protoplasm is unable to secrete the necessary diastatic ferment.

My experiments appear to establish the fact that a diastatic ferment is present in green leaves. The following description of a single typical experiment will give an idea of the methods adopted and of the nature of the results obtained:

500 grmes. of grass (with some Clover and *Achillea* intermixed), cut from a lawn, were well triturated in a mortar with 500 cc. distilled water: the mass was pressed through a strainer and a turbid slightly acid liquid, the leaf-extract, was obtained. In the afternoon, about four hours later (4.30 p.m.), the following mixtures were made in six jars, and were allowed to stand on the laboratory table till 6.45 a.m. the following morning, when they were all boiled at once; they were

<sup>1</sup> Comptes rendus, t. xcix, 1884.

#### 410

Notes.

then decolourised, and the presence of sugar and its amount in each was volumetrically determined by means of carefully prepared Fehling's solution:

Jar	- 2	No.		Nature of Mixture.						Amt. of Sugar (estd. as 1					trose).
														per 1	00 ce.
I		-5	o cc.	extrac	t + 5	o cc.	sta	arch-solut	ion		•			.0793	grme.
2	;	- 5	o cc.	extrac	ct (ba	niled)	+	"			•	•	•	.0450	,,
3	3	-5	o cc.	extrac	:t + 5	o cc.		"		+	thymol	•	•	.0740	,,
4	ŀ		"	,,	+	,,		,,		+	boracic	acid	·5 grme.	.0690	22
5	;	-	"	,,	+	,,		distilled	water	r	•	•		.0444	"
6	5	-	,, st	arch-s	oluti	ion +	,,	"	,,		•	•		no tra	.ce.

The starch-solution used was obtained by boiling  $\cdot 5$  grme. of starch with 100 cc. of dist. water; the liquid was allowed to cool and settle in a closed vessel, and only the nearly clear supernatant portion was used.

The activity of the ferment is represented by the difference between the amount of sugar in the leaf-extract to begin with, and the amount ultimately found in the mixture of leaf-extract and starch-solution: thus, from Nos. 2 and 5 it appears that the amount of sugar originally in the extract was about  $\cdot 045$  per cent., so that the amount of sugar formed from the starch-solution by the ferment in No. I is about  $\cdot 034$ per cent. It may be objected that this represents a very small amount of diastatic activity, so small indeed that it may be neglected as a factor in the almost wholesale conversion of starch into sugar which takes place in the living leaf; but this objection is deprived of weight by the consideration that, though the amount of ferment which can be extracted from leaves at any given moment is so small, yet the ferment is doubtless being constantly secreted, so that the total amount secreted during a night, for instance, would suffice to effect the observed conversion of starch into sugar.

In two cases (*Rheum hybridum*, *Daucus Carota*) somewhat peculiar results were obtained. In the former, the mixture of leaf-extract and starch-solution was found to contain less sugar  $(\cdot 1587^{\circ}/_{\circ})$  than the mixture of leaf-extract and distilled water  $(\cdot 2702^{\circ}/_{\circ})$ , after standing for 24 hours: in the latter also the mixture contained less sugar  $(\cdot 1052^{\circ}/_{\circ})$  than the leaf-extract diluted to the same bulk  $(\cdot 1250^{\circ}/_{\circ})$ . It would appear that, in these cases, the added starch was not attacked at all: hence the question arises as to the explanation of these varying amounts of sugar. An explanation is suggested by the further observation, made as a control-experiment in the case of *Daucus*, that

## Notes.

an equivalent quantity of diluted leaf-extract which was boiled at the beginning of the experiment contained only about  $\cdot 08^{\circ}/_{\circ}$  sugar. Since the unboiled leaf-extract, whether diluted with water or with starch-solution, contained more sugar than the boiled leaf-extract, it is clear that some amount of starch must have become converted into sugar; and it is also clear that, since this increase of sugar is most marked in the leaf-extract to which no starch-solution had been added, it must be due to the conversion of starch already present in the cells of the leaf. The presence of added starch appears to interfere with the action of the ferment upon the leaf-starch.

I have assured myself that the substance which reduces the Fehling's solution is really a sugar; but it does not appear to be maltose, as might be expected, for it is not dextrorotatory though it seems to be fermentable. Further details are given in the paper which I am preparing to be read at the meeting of the British Association at Cardiff.

S. H. VINES, Oxford.

### 412



Vines, Sydney Howard. 1891. "On the presence of a diastatic ferment in green leaves." *Annals of botany* 5, 409–412. <u>https://doi.org/10.1093/oxfordjournals.aob.a090647</u>.

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