with 7 (figure, h) are without it. While meiosis-I is reductional, meiosis-II is equational both for the autosomes and the sex-chromosomes.

Allodahlia macropyga (Westwood) has the lowest number of chromosomes of any species belonging to the family Forficulidae that has been studied cytologically so far. Thus, A. macropyga differs markedly not only from the rest of the forficulids but also from its congeneric forms in having 15 (16) chromosomes while the others have 24^{2-10}, except F. smyrnensis which has only 21 chromosomes in the male[11]. This significant difference in the number of chromosomes further favours the exclusion of A. macropyga from the genus Forficula and its inclusion in Allodahlia, as has been done by Burr[13], Kapoor[14] and many others on taxonomical grounds. However, to establish the modal number of chromosomes for the genus Allodahlia, more species are required to be worked out cytologically.

The unique feature of A. macropyga, is the possession of an XO type of sex-determining mechanism, which has not yet been reported in any of the Dermaptera, except that White[15] made a passing reference to the existence of XO-males in an Australian species of the genus Labidura studied by Webb (unpublished). By contrast, the occurrence of XY/XX$^{2-10}$, XXY/XXX$^{5-8}$ and XYY^{8} has been recorded in a number of species of the earwigs.

Henderson[7] pointed out that sex-determination in earwigs is not of the X : autosomal balance type as found in Drosophila, but of the type found in mammals where the Y-chromosome has male determining activity. His conclusion was based on the maintenance of the male phenotype in the individuals of F. auricularia, which possess XY, X_1, X_2, Y or XYY sex-chromosomes. Since an XO-male mechanism exists in A. macropyga, it seems to be more adequate to suspect determination of the X : autosomal balance type as in Drosophila.

13 M. Burr, in: Fauna of British India, Dermaptera, p. 151. 1915.
14 V. C. Kapoor, Agra Univ. J. Res. (Sci.) *16*, 28 (1967).
15 M. J. D. White, Chromosoma, Berl. *34*, 183 (1971).

Tuber formation and protein content in some wild cassava (Mandioca) species native of central Brazil[1]

N. M. A. Nassar and C. P. Costa

Instituto de Ciencias Biologicas, UFG, Goiania (Brazil), and Escola de Agronomia e Veterinaria, UFG, Goiania (Brazil), 14 February 1977

Summary. Screening for protein content in some wild species of Manihot showed 2 of them to have a notably high percentage of protein on dry matter basis. Moreover, one of these high-protein wild species was found to be extremely sweet.

Cassava, a staple crop that takes the 7th rank all over the world, represents an inadequately explored source for nutrition. Its ability to grow in sub-optimal conditions offers it a competitive superiority over all other staple food crops in underdeveloped nations. Cassava has various food forms which are well established in the consumption habits of the people but which are unfortunately characterized by their low protein content. If varieties with higher protein content could be developed, this would enhance the value of cassava as a food or/and animal feed. Efforts have been made in the past to increase the protein content of cassava roots by interspecific hybridization with a wild species known for its higher protein content[2]. This raised an increasing interest in looking for wild species, collecting them and screening them for protein content.

Among some wild species collected from Goias state, Brazil, 4 species were shown to form tubers. These species were screened for tuber formation, fibre and protein content. They are: M. oligantha pax emend. Nassar subsp. nesteli, collected from Cristalina (figure 1); M. tripartita Muell., collected from Serra Dourada, municipal Goiania (figure 2); M. zehntneri Ule, collected from Goianesia (figure 3), and M. anomala Pohl, collected from road Goiania–Inhumas (figure 4). These species differed largely in tuber formation pattern and tuber content. M. oligantha subsp. nesteli forms abundant cylindrical tubers, superficial, about 10.0 to 30.0 cm distant from ground surface, external color of tubers is dark brown, surface is rough, cortex is white. M. tripartita forms extremely globosus-shaped tubers, deep in the ground at a distance of more than 50.0 cm from ground surface, external color is light brown, surface is smooth, cortex creamy. M. anomala forms superficial tubers distant about 20.0–30.0 cm from ground surface, oval-shaped, with rough surface and light brown yellow color, cortex is creamy. M. zehntneri forms cylindrical to oval tubers, very deep in the ground, at a distance of about 50.0–70.0 cm from ground surface, external color is dark brown, has white cortex and rough surface.

Protein and fibre were estimated in tubers according to AOAC[3] procedure. Contents were shown as follows.

Average protein and fibre content of wild cassava species on a percent dry matter basis

Species*	Crude protein	Crude fibre
M. oligantha subsp. nesteli	7.10 ± 0.58	26.67 ± 4.86
M. tripartita	6.88 ± 1.48	33.48 ± 6.36
M. anomala	3.74 ± 0.63	23.44 ± 4.82
M. zehntneri	3.06 ± 0.82	21.52 ± 4.84

*20 tubers of each species were analyzed and replicated 4 times.

The composition of cassava as reported in the literature is somewhat variable. This variation comes from the fact that bitter cultivations differ from sweet ones, not only in the amount of HCN they contain, but also in the proportion of nutrients (according to Bolhuis[4], cultivars with roots containing less than 50 mg of HCN per kg are considered sweet). However, many reports state that crude protein dry matter ranges from 2.2 in sweet cassava to

2.7% in bitter cultivars, fibre percentage ranges from 3.1 to 10.3%[5,6]. One obviously finds notably high percentage of protein in the first 2 screened wild species in comparison to cultivated cassava. Some reports have referred to high protein percentage in some cassava cultivars which reach 6 or 7%[5,7], but indeed this subject is very doubtful since estimation of total nitrogenous matter must be viewed with caution because it is not certain whether the breakdown products of cyanogenic glucosides enhance the total nitrogen content or not. Narty[8] showed that the hydrolytic products of glucosides are incorporated into amino acids for protein synthesis in cassava. Therefore, it is not unlikely that the reported cultivars of high nitrogenous content turn out to be nothing else than bitter cultivars with high glucoside content. The one variety attracting attention in the screened wild species is M. oligantha subsp. nesteli due to its high protein content combined with a very low level of HCN[9]. The senior author saw cows and horses eat greedly the vegetative parts and

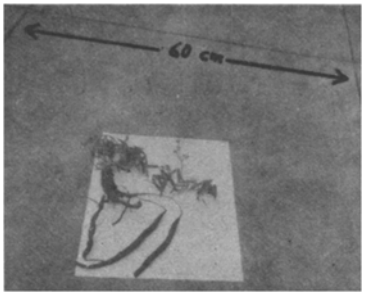
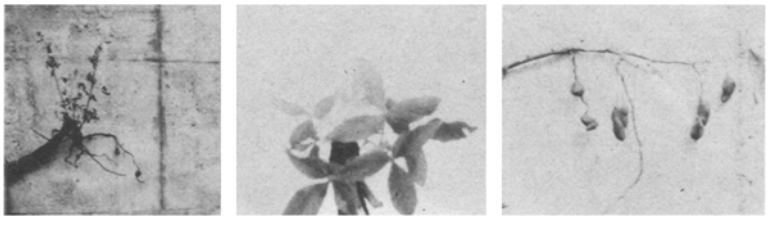


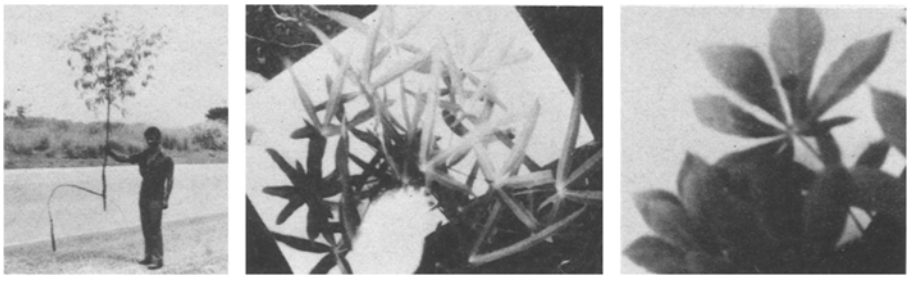
Fig. 1. M. oligantha subsp. nesteli, whole plant, with inflorescence on the right side.

1 This work is being carried out with the aid of a grant from the International Development Research Centre, Ottawa, Canada.
2 G. G. Bolhuis, Euphytica *2*, 107 (1953).
3 AOAC (Official methods of Analysis of the Association of Agricultural Chemists), 11th ed., 1075 p. Washington 1970.
4 G. G. Bolhuis, Neth. J. Agr. Sci. *2* (3), 176 (1954).
5 Anonumous 1968. Tabla de composición de pastes, forrajes y otros alimentos de Centro-América y Panamá (tables of composition of pasture grasses, forage plants and other food material of Central America and Panama), INCAP Publ. No. E-440.
6 V. A. Oyenuga, Nigerian feedstuffs, University College, Ibadan (1955).
7 P. Ammann, Acad. Sci., Paris *170*, 133 (1920).
8 F. Narty, Phytochemistry *7*, 1307 (1968).
9 N. M. A. Nassar, unpublished paper (1977).



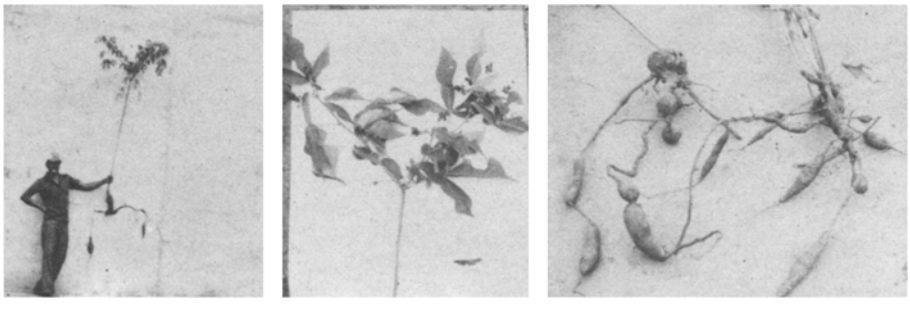
a *b* *c*

Fig. 2. M. tripartita. *a* Whole plant, *b* leaves shape, *c* tuber formation pattern.



a *b* *c*

Fig. 3. M. zehntneri. *a* Whole plant, *b* and *c* different shapes of leaves.



a *b* *c*

Fig. 4. M. anomala. *a* Whole plant, *b* leaves and inflorescence shape, *c* tuber formation pattern.

tubers of this species when grazing in its natural habitat. In literature, there are 2 other wild Manihot species which had been reported to have high protein content; M. melanobasis[10] and M. saxicola[11], but there is no reference to their HCN content; consequently, the authors have no idea how much the hydrolytic products of glucosides interfers with the total estimated crude protein. From the first instance, it seems logical to find wild cassava with high protein content, since human selection has aimed continually to select for tuber size and less fibre, without paying attention to protein content. This could lead to discarding protein-producing genes from the cultivated varieties.

10 O. L. Jennings, Euphytica *8*, 157 (1959).
11 J. Lanjouw, Recl Trav. bot. néerl. *36*, 542 (1939).

Hydrogen peroxide generation in *Trypanosoma cruzi*[1]

A. Boveris[2] and A. O. M. Stoppani[2]

Universidad de Buenos Aires, Facultad de Medicina, Instituto de Química Biológica, Paraguay 2155, 1121 CF, Buenos Aires (Argentina), 31 March 1977

Summary. Homogenates from T. cruzi epimastigotes produced 3.4 pmoles H_2O_2/min 10^6 cells, as detected by the cytochrome c peroxidase assay. Addition of NADH or NADPH increased H_2O_2 production by a factor of 3 and 5, respectively. When supplemented with NADH and NADPH, the mitochondrial, microsomal and supernatant fractions produced H_2O_2, the soluble fraction and the mitochondrial membranes being apparently the main generators of H_2O_2. The epimastigote homogenates showed cyanide-sensitive superoxide dismutase activity, equivalent to 0.28 μg bovine superoxide dismutase per mg homogenate protein.

Production of potentially toxic intermediates of oxygen reduction in cells and tissues was early suggested by Gerschman et al.[3,4]. Experimental confirmation of the biological formation of O_2^- and H_2O_2 was later provided by Chance et al.[5] and Fridovich[6]. The epimastigote (culture form) of Trypanosoma cruzi, the agent of Chagas disease, contains peroxidase[7,8], thus suggesting that H_2O_2 is a normal metabolite in this organism, but so far no direct evidence of H_2O_2 formation has been offered. Hydrogen peroxide is toxic for trypanosomes and, consequently, the study of H_2O_2 metabolism holds potential pharmacological interest, since an increased intracellular level of H_2O_2 may be lethal for T. cruzi[9,10].

Materials and methods. The Tulahuen strain of T. cruzi was grown as described before[8]. Epimastigotes were disrupted by freezing (at −16 °C) and thawing 3 times. The cell suspension was homogenized by several passages through a hypodermic needle, gauge No. 24, attached to a syringe. The homogenates were suspended in 0.23 M mannitol, 0.07 M sucrose, 1 mM EDTA, 10 mM Tris HCl, pH 7.2, at 8.0 mg protein/ml and fractionated in the Sorvall RC-2B centrifuge at 4 °C. The fractions obtained were: a) the nuclear-flagellar fraction (sedimented at 480 × g for 10 min; the fluffy layer was reincorporated to the supernatant); b) the mitochondrial fraction (sedimented at 12,000 × g for 10 min); c) the microsomal fraction (sedimented at 105,000 × g for 45 min); d) the supernatant.

NADH, NADPH, xanthine, horseradish peroxidase (EC 1.11.1.7; HRP) type VI, xanthine oxidase, bovine superoxide dismutase and D-glucose oxidase were purchased from Sigma Chemical Company. Cytochrome c peroxidase (EC 1.11.1.5; CCP) was prepared as described by Yonetani[11].

The rate of H_2O_2 generation was determined spectrophotometrically, by measuring the formation of the CCP-H_2O_2/complex (reaction 1)

$$CCP + H_2O_2 \rightarrow CCP\text{-}H_2O_2 \quad (1)$$

and the HRP-H_2O_2 complex (reaction 2)

$$HRP + H_2O_2 \rightarrow HRP\text{-}H_2O_2 \quad (2)$$

as described previously[12,13]. Superoxide dismutase was determined on the basis of its ability to inhibit the O_2^- dependent adrenochrome formation from epinephrine, using the xanthine oxidase reaction as source of O_2^-, as described in Cadenas et al.[14]. Production of O_2^- by beef heart submitochondrial particles was measured as described by Cadenas et al.[14].

Protein content of cell suspensions and fractions was determined by the biuret method[15] in the presence of 0.2% sodium deoxycholate. One mg of epimastigote total protein corresponded to 1.7 mg dry weight, to 12.3 mg wet weight and to 77×10^6 cells.

Generation of hydrogen peroxide by T. cruzi fractions*

Fraction (percent of the homogenate total protein)	Substrate	H_2O_2 generation (nmoles/min mg protein)	
		Antimycin omitted	Antimycin added
Nuclear-flagellar (20)			
	NADH	0.77	–
	NADPH	0.14	–
Mitochondrial (40)			
	NADH	2.35	2.30
	NADPH	0.75	0.70
	Succinate	0.00	0.00
Microsomal (7)			
	NADH	1.18	–
	NADPH	0.45	–
Supernatant (33)			
	NADH	4.7	–
	NADPH	6.0	–

*All samples were made of 130 mM KCl, 20 mM phosphate buffer (pH 7.2), 0.6 μM HRP and 0.2–1.0 mg protein/ml. 40 μM NADH (or NADPH), 7 mM succinate and 1–2 μM antimycin were added where indicated.

Results. Trace A in figure 1 shows that addition of CCP to respiring epimastigotes suspended in saline medium did not reveal formation of H_2O_2, despite the fact that limiting amounts of H_2O_2 formed by an extracellular H_2O_2