

BIOLOGICAL ACTIVITY OF THE SHRUB Boscia sergalensis (PERS.) LAM. EX POIR (CA.PARACEAE) ON STORED GRAIN INSECTS

D. SECK, 1,* G. LOGNAY, 2 E. HAUBRUGE, J.-P. WATHELET, 2 M. MARLIER, 2 C. GASPAR, 1 and M. SEVERIN

¹Unité de Zoologie zénérale et appliquee ²Unité de Chimie genérale et organique Faculté des Sciences agronomiques sage des Déportés, 2, B-5030 Gembloux, Belgum

(Received February 25, 1992; accepted October 13, 1992)

Abstract—1. Jogical activity of leaves, fruits and extract of the African surub Boscia seneral crisis (PERS.) LAM, ex Poir, was evaluated against five stored-grain insect. When added to cowpeas at 2.4% (w/w), fresh ground fruits and leaves coved 80-100% mortality in Callosotruchus maculatus it inabilits and significancy reduced both emergence and damage of the Exprogeny. Acetone fruit extract exhibited a potent furnigant effect on Prostephanics (rot catus HORS). C. maculatus, and Suotroga cerealeila OLIV, with LTs values of 3.—2.3, and below 1.5 hr, respectively. LCs determination for B. senegalensi in its and leaves as well as pure methylisothiocyanate (MITC) on Tribolic in castaneum HERBST, Sitophilus reamais MOTSCH, and C. maculatus stored a differential response of the insects to plant parts of MITC. Quantitative in sage of Boscia active components and LCs, values obtained for the plant dissues, compared to those of pure molecules, indicate that the biological activity of B, senegalensis is due to the liberation of MITs from a glucosinol in precursor glucocapparin contained in Boscia Iruits and leaves.

Key Word Coscia senegalensis (PERS.) LAM, ex Poir ; glucosmonates, methylisoth canate, Vigna unguiculata (L.) WALP., Callosobi acnas macalanis (F.), 1 cephanus truncatus HORN., Stophilus zeamais (MO) 5CH i; Stotroga ce calella (OLIV.), Triboluon castaneum HERBST coreope ta

^{*}To whom correspond - a should be addressed

INTRODUCTION

Insect infestation of stored : reduction of commercial va several control measures have but are often frequently use insecticide resistance is als (Georghiou, 1990). This 8 synthetic pesticides. Botani able option. The effectiven Singh et al., 1978; Jacobso 1985; Lognay et al., 1991:

Wc investigated the ! LAM. (Capparaceae), a pla Senegal (Bille and Poupon against stored grain insects active components of this p

in causes weight and quality losses that lead to a and seed germination. Fo reduce this damage, been taken, which mostly involve chemical methods. Synthetic insecticides a . not only a drain on the farmer's meager resources beyond permissible safe limits. The incidence of a growing problem. Resistance to one or more insecticide(s) has been reped in at least 500 species of insects and inites ation has increased the need for alternatives to insecticides are a less expensive and biodegrad , of raw parts and plant extracts against storedgrain insects has been desc. Bed by many authors (Jotuani and Sircar. 965; 1983; Golob and Webley, 1980; Grainge et al., ck et al., 1991; Haubruge et al., 1989). ogical activity o t Boscia venegalensis (PERS.) hat is found throughout north-central and northern 1972), where it is traditionally used by farmers , 1 addition. we isolated and identified biolog cally

HODS AND MATERIALS

Plant tnaterial was ra-Our material has been ident de Belgique (BR) as B. sci November 1990, Fruits and 1991.

Four experiments wer (trial 3), and pure molecul test insects. Callosobrucha alella OLIV. (Lep., Gelec trychidae), Tribolium cast zeamais MOTSCH. (Col. ditions (32" ± 2°C and carried out under these co. grade.

Trials I and 2. Thes leaves (FGL), fresh entit ground fruits (FGF). The unguiculata (L) WALP (v in trial |: and ().5-2% for

imly harvested in the region of Thies (Senegal). ed and deposited at the Jardin Botanique National alensis. Leaves used for trial I were harvested in aves used for trials 2 and 3 were collected in May

inducted with plant parts (trials I and 2), extracts trial 4) using five stored-grain insect species. The naculatus (F.) (Col., Bruchidae). Sitotroga verelac), Prostephanus truncatus HOKN. (Col Bosum HERBST. (Col., Tenebrionidae), Sitophilus arculionidae), were reared under controlled con-± 10% relative humidity.) Bioassays were also ons. All chemicals and solvents were of ana vtical

rst two trials were performed with fresh ground aves (FEL), dry leaf powder (DLP), and fresh t materials were added to cow pea seeds, Vigna Black Eyed) at 1-32% for FGL, FEL, and DLP and FGL in trial 2. FGL and FGF were prepared

using a small laboratory and then passed through a 2 0 g of cowpeas were reconcentrations in Petri d old *C. maculatus* adults 10 insects. Each treatmer Mortality assessment wa when F, adults started to 10 days. Numbers of ho damage was calculated.

Data were subjected the means (Duncan's mul-

Trial 3. Fifty gram a Waring blender and kethen filtered and concent: a *B. senegalensis* acetor by volume of water.

Bioassays were care; mill of BAFE were depoin another desiccator uses evaporated completely as were then introduced in experimental conditions tality readings were recelected, of the insects with probital and calculating the second of the care of

Analysis of volatile (100 g) were steam-diextracted three times will drated with anhydrous the solvent at 38°C, an using two types of columns a CP-Wax 52CB (Chrompack; carrier gasto 240°C; apparatus; H Sil 8 CB (25 m long; Carrier gas was helium 5°C/min; cold "on-columparatus; Carlo Erba N

Analysis of glucos. galensis FGF, FGL, an

<Moulinex) For 5 min. DLP was similarly; round 3-mesh laboratory sieve. Following this procedure, oughly mixed with plant material at the desired (φ 90 mm) and infested with 10 unsexed f day-reated cowpea seeds were similarly infested with including the control, was replicated five times de after one to two days. Twenty-two days later erge, insects were removed daily and counted for and unholed seeds were also counted and percent</p>

analysis of variance followed by a comparison of z-range test) at P = 0.05.

Boscia fruits were ground with 200 ml acctone in τ 2 hr at room temperature. The supernatants were under vacuum to a final volume of 50 ml to obtain it extract (BAFE) containing approximately 30%

out in 825-ml scaled glass desiccators in which 5 Five milliliters of acetone were similarly proetted control. After 3 hr at room temperature, the solvent control. One hundred adults of different species desiccators, which were closed and placed under r various time exposures, from 1,5 to 12 hi, morfor each treatment. The time required to ki! 50% en determined by transforming mortality data to thal times (LT) (Snedecor and Cochran, 1967). Boscia senegalensis leaves. Freshly ground leaves for 45 min and the aqueous distillate (900 ml) 00 ml diethylether The ether solution was dehyn sulfate, concentrated to 4 mt by distillation of ty analyzed by gas-liquid chromatography GLC) inder the following conditions: The polar column long, 0.32 mm ID, 0.2 μm film thickness) from relium at 100 kPa; temperature program from 30 'on-column' injector and FID detector maintained Packard HP 5880 The apolar column was a CP im ID, 0.2 μ m film thickness) from Chrompack: kPa; temperature program: from 3 0 to 240 C at injector and FID detector maintained at 300°C: 5160.

es. This glucosinolates were analyzed in B sense EE by reverse-phase HPLC after enzy matic desul-

380 SECK , M

fation according to the official EEC method (European Economic Community, 1990). Identification of BAFE glucosinolates was performed by GC-MS analyses of trimethylsilylated molecules and by examination of their degradation products liberated enzymatically under controlled conditions. For GC-MS investigations, glucosinolates were enzy matically transformed to desulfoglucosino lates and trimethyl-sily lated for 20 m i n at 110°C with 50 μ l of a reagent containing N-methyl-N-trimethylsilyltrifluorobuty ramide, 5% methylimidazole in acetone and trimethylchlorosilane 30 : 15 3 (v/v/v). The chromatographic conditions were as follows: Column SE-53 (25 m long, 0.35 mm ID, 0.2 μ n film thickness) was from Macherly-Nagel; carrier; helium at 50 kPa; temperature program; SO-780°C at 20°C/min; cold "on-column" injection. The mass spectra were recorded in the El mode on a Nermag R 10-10C spectrometer. 70 eV source at 130°C, interface at 280°C, mass range scanned from 100 to 800 amu coupled to a Delsi DI-700 gas chromatograph.

For enzymatic degradation of *B. senegalensis* glucosinolates, 0. Imlacetate buffer (pH 4.5) and 50 μ l of 10 mg/ml buffered solution of thioglucosidase (EC 3.2.3.1), purified from *Sinapis alba* L. according to Appelqvist and Josefson (1967), were added to 0. Iml of residual aqueous phase from BAFE or to a solution of pure methylglucosinolate (glucocapparin, Roth ref. 7485). After 24 hr. the degradation products were extracted with 2 ml diethylether and analyzed hy GLC on polar and apolar stationary phases. Methylisothiocyanate (MITC) from BAFE and glucocapparin were identified by comparison of their retention times with that of a pure reference (Sigma ref. M8632)

Detection of MITC in Volatiles Liberated during Biotests. Acetone solutions of BAFE (5 ml) were introduced into conical flasks. After evaporation of acetone, the vessels were hermetically closed and left for 24 hr at $32 \pm 2^{\circ}C$. Headspace sampling was performed by purging the apparatus with nitrogen for 30 min. The volatiles were collected in 3 ml diethylether maintained in a cryogenic trap at $-20^{\circ}C$. Ether solutions were submitted to GC-MS in the aforementioned conditions except that the temperature program rate was fixed at $5^{\circ}C$ /min. MITC was identified on the basis of retention and mass spectral data. The mass spectra were compared with EPA-NIH and Wiley libraries.

Trial 4 . To study the dose-mortality responses to *B. senegalensis* tissues and MITC, various amounts of FGF and FGL from () to X g/liter (w/v) and pure MITC from 0 to 3 mg/liter (w/v) were deposited in 750-ml glass desiccators containing 25 adults of each insect species. In four replications. After 24 hr under the experimental climatic conditions, insects were transferred to clean Petri dishes and maintained in controlled conditions until the next day for mot tality readings as indicated by Busvine (1981). Data were subjected to probit analysis (Finney, 1964). Log dose-probit line was analyzed for goodness of fit by the chi-square test (Busvine, 1981), followed by computation of LC₅₀ values for each material.

RESULTS

Trial 1

Mortality. FGL at a concentration of 4% (w/w) caused 100% mortality after 24 hr. At 2%, 55.6% of the insects died within 48 hr, and at 1%, mortality ranged from 7.4 to 11.1% in one to two days. FEL scored 0–18.5% mortality and DLP 3.7-40.7% within 24-48 hr (Table 1).

Progeny. FGL completely inhibited the production of *C. maculatus* progeny at 2%, but at 1%, 21.6 adults emerged. At the same time, progeny ranged from 36.2 to 87.2 adults for FEL and from 40.2 to 53.2 for DLP (Table 1).

Table 1. Biological Activity of B. senegalensis Fresh Ground Leaves (FGL). Fresh Entire Leaves (FEL) and Dry Leaf Powder (DLP) to C. maculatus Adules, F_1 Progeny, and Damage (Means)"

		Corrected mortality (%)"			
Treatment	Conc (%, w/w)	24 hr	48 hr	F ₁ progeny	Damage (%)
FGI.	1	7.40	11 le	21.6ab	22 Ob
	2	63.0b	55.6b	0.0h	0.0b
	4	100.0a	100a	0.0b	0.0h
	8	100 Oa	100a	0.0b	0.0h
	16	100.0a	100a	0.06	0.06
	32	100.0a	100a	0.0b	0.0h
	Control			61.6a	70.3a
FEL	1	7.4a	18/5a	36.2b	49 1a
	2	(0,0)a	? 4ab	43.6ab	36.9a
	4	O.Oa	7.4ab	69.0ab	59.8a
	8	0.0a	0.0b	87.2a	64.8a
	16	(0,0)a	0.06	62.4ab	52.9a
	32	().()a	().()b	71.8ab	60.5a
	Control			61.6ab	56.3a
DLP	I	11.1a	37.0a	53.2a	67.5a
	2	3.7a	18.5a	47.6a	44.4abc
	4	t1. la	25.9a	51.0a	52.4abc
	8	11.1a	33.3a	49.8a	33.6bc
	16	14.8a	33.3a	40.2a	39.7abc
	32	11.1a	40.7a	45.8a	30.8c
	Control			45.0a	65.1ab

[&]quot;Means followed by the same letter within a column of each treatment are not significantly different at the 5% level (Duncan's multiple-range test).

[&]quot;By Abbott's (1925) formula.

382 Seck et al.

FGL significantly reduced *C. maculatus* progeny, compared to FEL and DLP (Figure 1).

Damage. FGL gave 100% protection at 2.4 and 23 % damage at 1 % In the same conditions. damage was 36.9-64.8% for FEL and 30.8-67.5% for DLP (Table 1).

Trial 2

Mortality. After 72 hr at concentration of 2%, mortality was 93.6% for FGF and 24.8%. for FGL. After 4X hr, it ranged from 3.X to 79.8% for FGF compared to O-24.2% for FGL. After 24 hr, mortality ranged from 3.X to 73.8% for FGF and from 0 to 8% for FGL (Table 2).

Progeny. At 2%, FGF showed no adult emergence while 7.8 and 133.8 adults emerged, respectively, in FGL and the control treatment. At 1%. 12 adults emerged from FGF compared to 104 for FGL (Table 3).

Damage. A t 1% concentration, damage was 8.7% for FGF and 63% for FGL. At 2% concentration. FGF gave 100% protection, while 6.1 damage was noted in the FGI. treatment (Table 3).

Trial 3

 LT_{50} values were 2.3 hr for *C. maculatus* and 3.8 hr for *P. truncatus* (Table 4). For *S. cerealella* all adults died within 1.5 hr.

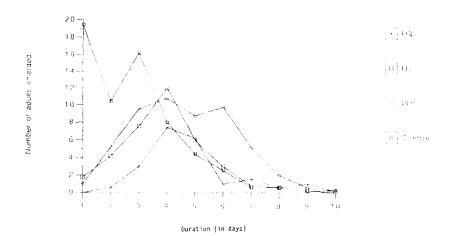


Fig. 1. Emergence pattern of C, maculatus from cowpea seeds treated with fresh ground leaves (FGL), fresh entire leaves (FEL), or dry leaf powder (DLP) of Boscia senegalensis at 1% conc. (w/w).

Table 2. Comparative Toxicity of *B. senegalensis* Fresh Ground Fruits (FGF) and Leaves (FGL) to *C. maculatus* (Means)^d

		Corrected mortality after (*		
Treatment	Conc (%, w/w)	24 hr	48 hr	72 hr
FGF	0.5	3.8b	3.8c	1.9c
	1	6.4b	24.9b	35.9h
	2	73.8a	79.8a	93.6a
FGL	0.5	0.0a	0.0b	23.4a
	I	3.8a	5.8ab	13-0a
	2	8.0a	24.2a	24.8a

[&]quot;Means followed by the same letter within a column of each treatment are not significantly different at the 5% level (Duncan's multiple-range test).

Table 3. Effect of Cowpea Treatment with B. senegalensis Fresh Ground Fruits (FGF) of Leaves (FGL) on C. maculatus Emergence and Damage in Cowpeas (Means) a

	<i>(</i>)	Number of	Percentage	Reduction (%)	
Treatment	Conc. (%, w/w)	emerged adults"	Damage (%)	Emergence	Damage
FGF	0.5	3.4a	22.7a	68.2a	73 Oa
	1	1.6b	8.7a	90.7a	89.6a
	2	0.0e	. 0.0a	100a	100a
FGL	0.5	4.2a	46.0a	41.7b	45.2b
	l	4.6a	63.0a	22.3b	25.0b
	2	1.8b	6.16	94.5a	92.7a
Control		4.9	84.0		

[&]quot;Means followed by the same letter within a column of each treatment are not significantly different at the 5% level (Duncan's multiple-range test).

Trial 4

Acute Toxicity of B. senegalensis Fresh Ground Tissues and Pure MITC. FGF, FGL, and MITC exhibited a differential acute toxicity against three stored-grain beetle species. LC_{50} values (in grams ground matter per liter volume) ranged from 1 to 4.23 for FGL and from 0.42 to 1.75 for FGF (Table 5). LC_{50}

^bBy Abbott's (1925) formula.

[&]quot;Log (numbers).

384 Seck et al

Table 4. Lethal Time (LT) Values of Two Stored-Grain Insects Exposed to Vapors of *B. senegalensis* Fruit Extract (BAFE)

Species"	Stope ± SE	LT _{S0} (95% FL*), hr
C. maculatus P. truncatus	$\begin{array}{c} 4.47 \pm 1.71 \\ 4.21 \pm 1.16 \end{array}$	2.31 (1.01-5.28) 3.80 (1.92-7.54)

[&]quot;One-day-old C maculatus; 2-week-old P. truncatus.

TABLE 5. ACUTE TOXICITY OF *B. senegalensis* Fresh Ground Fruits (FGF) and Leaves (FGL) to Adults of Three Stored-Grain Insect Species

		FGF	FGL		
Species"	Slope \pm SE = LC ₈₀ (95% FL) ⁶ = Slope \pm SE	LC ₅₀ (95% FL)"			
Tribolium castaneum Sitophilus zeamais		1.75 (1.63~1.86) 0.87 (0.80~0.94)	6.14 ± 1.38	4.23 (1.11-16.15)	
Callosobruchus maculatus	4.91 ± 0.75	0.42 (0.36-0.47)	6.15 ± 0.93	1.00 (0.88-1.09)	

[&]quot;Four replications of 20 insects were exposed to plant materials for 24 hr before they were transferred to Petri dishes and placed in controlled conditions (30°C, 70% relative humidity). Mortality was counted after 24 hr of pest exposure (Busvine, 1981).

for MITC ranged from 0.73 to 2.38 ppm according to insect species, with *C. maculatus* being the most susceptible (Table 6).

Chemical Investigations

GLC analysis of the volatiles from FGL showed one major peak (92 % of the total area) at retention times of Y.3 min and 6.5 min, respectively on polar and apolar columns, which corresponded to those of an authentic sample of MITC. The detection of MITC from B. senegalensis leaves (Figure 2) suggested that methylglucosinolate (glucocapparin) may be the main precursor of the insecticidal compound. Glucosinolate enzymatic degradation leads to several by products among which isothiocyanates predominate (Tookey et al., 1980). To test the aforementioned hypothesis, we analyzed the residual water phase of BAFE following a three-step procedure (HPLC, identification of glucosinolate by-product, and GC-MS). The HPLC retention time of pure desulfomethylglucosinolate (2 min) corresponded to that of the major peak detected in BAFE. On the other hand, the elution profiles liberated enzymatically from BAFE

[&]quot;Fiducial limits.

[&]quot;Grams per liter volume, with Fiducial limits.

Table 6. Acute Toxicity of Methyl Isothiocyana is to Adults of Three Stored-Grain Insect Species

Species"	Slope ± SE	LC ₅₀ (95% FL) ⁶
Tribolium castaneum	7.05 ± 0.58	2.38 (2.24~2.52)
Sitophilus zeamais	7.22 ± 1.18	1.25 (1.14-1.34)
Callosobruchus maculatus	4.81 ± 0.59	0.73 (0.64-0.82)

[&]quot;Four replications of 20 insects were exposed to plant materials for 24 hr before they were transfered to petri dishes and placed in controlled conditions (30°C, 70% relative humidity). Mortality was counted after 24 hr of pest exposure (Busvine, 1981).

"ppm, with Fiducial limits.

residual aqueous phase and methylglucosinolate are practically identical; MITC is distinguishable on the two chromatograms but is absent on the blank. The identitiention of glucocapparin in BAFE was finally validated by CC-MS A typical mass spectra is shown in Figure 3. The mass fragments at m/e = 103. 117, 137, 169, 204, 243, 271, 361 (base peak), and 451 were generated by the glucidic moiety of the molecule and were not of interest for the identification of the aglycone. Nevertheless, the ions recorded at m/e = 613 (M) $^{\circ}$. 598 (M-CH₃)', 524 (M-CH,-TMS) and 508 (M-CH₃TMSOH) indicated clearly a glucosinolate bearing a methyl radical. As for all other alkylglucosinolates, the intensities of these characteristic mass fragments were low The methylglucosinolate content of B. senegalensis material tested in trial 4 represented 23.6 \pm 0.8 μ mol/g fresh leaves and 38 \pm 1.2 μ mol/g fresh fruits (HPLC determination with sinigrin as internal standard). Headspace sampling conducted under the same conditions as trial 3 and trapping the volatiles in diethyl ether at - 20°C led to detection of MITC from the vapor phase. The total ion current (Figure 4) showed several peaks, among which was MITC (K, = 16.6 min). The molecule was unambiguously identified by comparison of its mass spectrometric pattern with EPA-NIH and Wiley libraries and also on the basis of GLC retention time.

DISCUSSION

This research has demonstrated a significant biological effect of *B. sene-galensis* plant parts and extracts. The evidence in support of these results was obtained from four experiments. First, B. *senegalensis* FGL (when added to cowpeas at 4% w/w) completely killed *C. maculatus* adults within 74 hr. inhibited the production of F₁, progeny and prevented bruchid damage. Under the same conditions. FEL and DLP had almost no effect. Second, comparative

386 SECK ET AL.

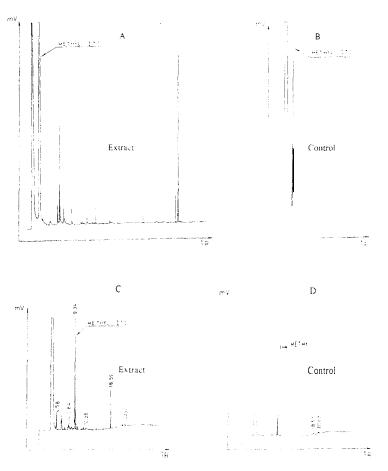


Fig. 2. Identification of methylisothiocyanate (MITC) from *Boscia senegalensis* leaves with apolar column (A and B) and polar column (C and D).

evaluation of FGF and FGL revealed that fruits were more toxic to *C. maculatus* and reduced both progeny and damage to a greater extent, than did leaves Third, BAFE exhibited a high f'umigant effect on three stored-grain insect species, which had a differential time-mortality response. Finally, we quantified the acute toxicity of *B. senegalensis* fresh ground fruits and leaves as well as pure MITC and obtained dose-mortality responses for three stored-grain beetles.

B. senegalensis is a shrub, growing up to 3 m high, that is frequently found on abandoned tennite mounds and on barren and fire-scorched soil of the Sahel. It is distributed from Mauritania to Niger, northem Nigeria, the northwest Cameroons, and across Aftica to Sudan and Ethiopia (Booth and Wickens, 1988).

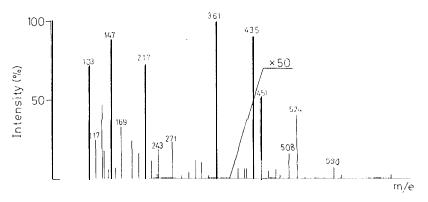


Fig. 3. Mass spectrum of silylated desulfoglucocapparin from *Boscia senegalensis* fruit extract.

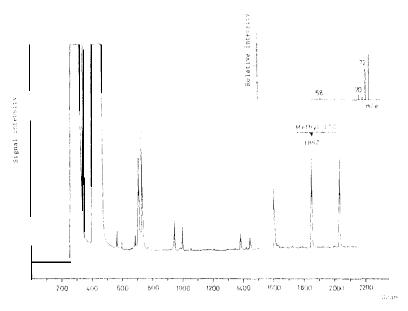


Fig. 4. GC-MS total ion current of the volatiles liberated during the bioassays of *Boscia senegalensis* fruit extract.

Leaves and fruits are used as human food and animal fodder (Bernus, 1979: Baumer, 1981; Maydell, 1983; Burkill, 198.5: Becker, 1983). The leaves, bark, and roots are widely used in northem Senegal for their medicinal properties (Kerharo and Adam, 1974; Hooth and Wickens, 1988).

388 Seck et al

Alzouma and Boubacar (1985) reported on the toxicity of *B. senegalensis* leaves from Niger, which also reduced *Bruchidius atrolineatus* Pic. and *C. maculatus* oviposition, but they gave no details about the active components. Kjaer et al. (1973) reported that *B. senegalensis* leafless twigs contained methyl and isopropyl glucosinoiates.

Our bioassays, performed by comparing *Boscia* tissues and MITC, indicate that *B. senegalensis* fruits, leaves, and MITC were toxic to insects at various levels, according to the species and the plant tissue. They also indicate, considering LC,, values l'or FGF. FGL, and pure MITC on the one hand, and the amounts of glucocapparin found in the plant tissues on the other hand, that Boscia fruits and leaves contained sufficient glucocapparin to liberate MITC at levels comparable to the LC₅₀ of the pure molecule.

These results indicate that in addition to its medicinal properties (Dalziel, 1948) and utilization as a famine food (Becker, 1986; Salih et al., 1991), B. senegalensis also has potential in stored-grain protection due to a potent fumigant effect on different insect species. B. senegalensis has been traditionally used by African fat-mers as a grain protectant, but the basis of its effectiveness has never been explained. We have shown that B. senegalensis biological activity is linked to the liberation of methyl isothiocyanate from a glucosinolate precursor, glucocapparin, contained in its fruits and leaves. As the plant grows spontaneously in some of the poorest areas of the world (mainly in the arid sahelian regions), this research suggests a natural insecticide from B. senegalensis as an alternative to synthetic pesticides in developing countries.

Acknowledgments—We are grateful to Mr. J.C. Gilson for drawing figures and Miss A. Van Meensel for typing the manuscript. Dr. J.-L. Hemptinne and two anonymous reviewers made valuable comments on the manuscript.

REFERENCES

ABBOTT, W.S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18:265-267.

ALZOUMA, I., and BOUBACAR, A. 1985. Effet des feuilles vertes de *Boscia senegalensis* (Cappardaceae) sur certains aspects de la biologie de *Bruchidius atrolineatus* et de *Callosobruchus maculatus*. Comm. Colloque sur les Légumineuses Alimentaires. Niamey, Niger.

Anonymous, 1990. Règlement CEE No. 1864/90. Graines oléagineuses—détermination des giucosinolates par chromatographie liquide à haute performance. J. Off. Commun. Eur. 1.170:27.

APPELOVIST, L.A., and JOSEFSON, E. 1967. Method for the quantitative determination of isothio-cyanates and oxazolidinethiones in digests of seed meals of rape and turnip rape. J. Sci. Food Agric, 18:510.

BAUMER, M. 1981. Rôle de *Boscia senegalensis* (PERS.) LAM. dans l'économie rurale africaine. Sa consommation par le bétail. *Rev. Elev. Med. Vet. Pays Trop.* 34(3):325-328.

BECKER, B. 1983. The contribution of wild plants to human nutrition in the Ferlo (northern Senegal)

**Agrofor. Syst. 1:257-267.