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Establishment of Productive Rangeland with High-Yielding Fodder Shrubs

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Table of Contents

Executive Summary: 3

Section I 4

A) Research Objectives 4

B) Research Accomplishments 4
- B.1. Trials on the nutritive value of fodder shrubs (South Africa) 4
- B.2. Field trials (South Africa) 5
- B.3. Selection (South Africa) 7
- B.4. Analyses of plant material (Israel) 8
- B.5. Analyses of plant material (South Africa) 9
- B.6. Field observations, Omer Fodder Shrub Introduction Site (Israel) 10
- B.7. Rooting of cuttings 11
- B.8. *In vitro* studies 13

C) Scientific Impact of Collaboration 24

D) Description of Project Impact 24

E) Strengthening of Developing Country Institutions 24

F) Future Work 25

Section II 26

A) Managerial Issues 26

B) Budget 26

C) Special Concerns 26

D) Collaboration, Travel, Training and Publications 26

E) Request for American Embassy Tel Aviv or A.I.D. Actions 28

Executive Summary

Rangeland-based livestock production is a principal source of income in semi-arid areas of southern Africa. Such rangeland is often degraded as a result of overgrazing and poor management. The goals of this program are to contribute to rangeland rehabilitation and improvement a) by introducing promising fodder species and sustainable land use principles, b) by selecting the most promising species and individual shrubs for the target areas (on the basis of agronomic and nutritional traits) and c) by making these superior shrubs available through clonal propagation.

In continuation of the first year's work, all aspects of the program were addressed this year.

F_1 seedlings, produced from seed of shrubs selected for palatability in earlier trials, were planted out for recurrent selection by grazing sheep as well as for studies of nutritive value. Shrub plantings and evaluations have continued at sites in five areas of the Northern Cape with different soil types and climatic conditions (SA). Among the parameters being evaluated are production, palatability and nutritional value (both countries). Preliminary studies of metabolizable energy (Israel) showed *Atriplex* species provide more metabolizable energy than *C. sturtii*. The ash content and crude protein content was also higher in *Atriplex*.

The influences of mechanical soil disturbance, stone mulches and water harvesting are being investigated (SA). Also, water absorbent polymers are being tested to improve soil water-holding capacity (SA).

A. nummularia, *A. glauca* and *Cassia sturtii* have been identified as priority exotic species in terms of palatability (SA). Browsing herbivores have shown a preference for *C. sturtii* over *A. nummularia* (both countries).

Improvements have been made in a protocol for rooting cuttings of *A. nummularia* and *C. sturtii*. Both species rooted better in hydroponics than in solid potting substrate; *A. nummularia* at the rate of 90%; *C. sturtii* at 23%. 100% of the rooted cuttings from hydroponics and from particulate potting mixes were hardened-off successfully.

Several efforts were made to improve micropropagation protocols. Semisolid MS medium was found to be best of those tested for *A. nummularia*, with 60-70% of the plantlets surviving hardening-off. As opposed to *A. nummularia*, we have thus far been unsuccessful in micropropagating *A. canescens*. With respect to *C. sturtii*, lethal problems due to metabolites produced in culture were alleviated by the use of WPM supplemented with activated charcoal. All cultures in this medium formed shoots and approximately 40% rooted. Work also proceeded along several lines toward propagation via callus and somatic embryogenesis, but there have not been any breakthroughs as yet.

Visits of the South African investigators to the BIDR and a period of training in tissue culture for a South African technician and a graduate student, scheduled for this year, were delayed to the third year. There has been as strong training component, with graduate students at both institutions involved in all aspects of the work. There has also been valuable collaboration with members of the farming community in field trials in South Africa.

Section I

A) Research Objectives

Rangeland-based production of livestock is a principal source of income for the populations of semi-arid areas of southern Africa. Overgrazing and poor management of rangeland has led to erosion and desertification. This program aims to facilitate the establishment of improved fodder reserves in such areas, composed of high-yielding perennial shrubs and trees, which would aid in establishment of a sound economic base for rural agrarian populations. Specific objectives are to:

1. introduce promising fodder species (shrubs and trees) to target areas in Southern Africa, and conduct performance and palatability trials.
2. introduce and test sustainable land use principles on plots of introduced fodder shrubs.
3. select the most promising species and individual shrubs on the basis of agronomic traits, palatability and nutritional tests, for cultivation on poor soils in areas where rainfall is irregular.
4. develop procedures for large-scale clonal propagation of the selected shrubs; establishing centers in South Africa for fodder shrub propagation and distribution.

The South African and Israeli research teams continued to address each of these goals during the second year of the program.

B) Research Accomplishments

B.1. Trials on fodder shrub nutritive value (South Africa)

Studies of the nutritive value of fodder shrubs continued, using, when necessary, facilities of the Agricultural Research Council. These have included evaluations of:

- the quality of edible plant material
- the energy supplementation of oldman saltbush, *A. nummularia*, including analysis of nutritive value, following the method used in Degen's lab

- the degradability and fermentation of components of fodder shrubs

Seed harvested from Hatfield Elite plants (selected for palatability in earlier trials by Verschoor and Malan) under bird netting has been used to produce F_1 seedlings. These have been established for recurrent selection by grazing sheep in the 2002/2003 season. The most palatable of these F_1 plants will be moved to a seed production block (under bird netting) on Hatfield for the production of F_2 seedlings.

The first batch of *Cassia* seedlings, to be planted in December 2002 has been prepared and more are being produced for an autumn planting at Hatfield. These plantings will become available for nutritive value studies as well as forage characterization.

B.2. Field trials (South Africa)

Mier

Surviving *Atriplex* species at this site have been evaluated for production, palatability and nutritional parameters, with particular reference to the effect of high alkalinity/salinity. *Cassia* seedlings have been planted for the first time at this Kalahari site and in the coming years these will be evaluated for adaptation, production and nutritive value under these harsh conditions. As an addendum, we have also planted *Harpagophytum* (an adapted indigenous species, commonly called Devils Claw), which is used for medicinal purposes and may play an important role in diversifying land use options for the rural people in the Kalahari.

Hotazel

We are evaluating at this site the possibilities of introducing indigenous fodder shrubs in combination with Devils Claw into the Kalahari Thornveld (Deep Kalahari sands, with more grass and trees under the relatively "high" rainfall conditions [400 mm, as compared with <200 mm at Mier]). It is hypothesized that such shrubs will improve the fertility and hence the growth rate of the *Harpagaphytum*, consequently improving the quality of grazing in the winter months. We are planning to establish trials using seed harvested in the 2001/2002 season and seedlings being produced in Pretoria, during the 2002/2003 season.

Kenhardt, the Nama Karoo

On the "Klipkoppies" farm we established autumn and spring plantings of:

(i) F_1 seedlings of Hatfield Elite Oldman Saltbush (OMS)

(ii) *Cassia*.

(iii) several tree species, in co-operation with the Department of Water Affairs and Forestry and

(iv) the standard drought tolerant crops, *Opuntia* and *Agave*, for comparison purposes.

(v) We have also made provision for planting *Sutherlandia* and *Tripteris* in the 2002/2003 season.

Pofadder, representative of Bushmanland

At this site we are evaluating the possibilities of introducing indigenous fodder shrubs in combination with Devils Claw into the Kalahari Thornveld (Deep Kalahari sands, with more grass and trees under the relatively "high" rainfall conditions [400 mm, as compared with <200 mm at Mier]). It is hypothesized that such shrubs will improve the fertility and hence the growth rate of the *Harpagaphytum*, consequently improving the quality of grazing in the winter months. We are planning to establish trials using seed harvested in the 2001/2002 season and seedlings being produced in Pretoria, during the 2002/2003 season. One of these will be set on "run-on" and "runoff" bare areas, to examine the influences of soil disturbance/water harvesting and protection against herbivory on the successful establishment of volunteer natives and seeded indigenous shrubs. A similar trial on bare areas was conducted using a tractor and mechanical ripper to disturb the soil and improve water infiltration.

There is a spring planting of *Cassia* and OMS using seedlings, untreated and treated seed in riplines, with and without protection. Already it is very evident that when planting palatable species (especially *Cassia*) steps must be taken to protect against herbivory. Although domestic animals are excluded from this area, porcupines, rabbits and antelope browsed even the protected *Cassia* plants, while the unprotected *Cassia* had been destroyed. Unprotected

OMS plants had also been browsed, but the degree of defoliation was only approximately 30 %, relative to 10 % on protected OMS and *Cassia* and 100 % on unprotected *Cassia*.

A spring establishment of two tree species, *Rhus* and *Ziziphus*, has been set on bare areas evaluating such interventions as water-harvesting from bare areas, stone mulches to reduce evaporative losses, water absorbent polymers to improve water holding capacity and use of thorn branches to protect plants against herbivory. In addition a product (DRI-WATER) allegedly used to good effect on mineland re-vegetation projects in the USA and SA is also being evaluated.

Kammieskroon

In this winter rainfall area of Namaqualand two sites, "Pendoornhoek", which receives about 200 mm, and "Olienfontein", which receives about 400 mm, have been planted with Hatfield Elite OMS and *Cassia* in the spring. A replication of this is planned for the autumn of the 2002/2003 season, when indigenous shrubs such as *Sutherlandia* and *Tripteris* in addition to exotic winter rainfall species such as Tagasaste (*Chaemecytisis*), Carob and Olive will also be planted.

B.3. Selection (South Africa)

Of the *Atriplex* species evaluated, *A. nummularia* is still ranked best and we are persisting with recurrent selection from within this species. This does ensure some variation within the species but avoids the pitfalls of homogeneity resulting from vegetative propagation. We do, however, have material propagated from single elite plants on which we can do more critical studies of intra-species variation.

We have also identified *Cassia sturtii* and *A.glauca*, as priority exotic species in terms of palatability. *Cassia* appears to be very uniform, offering little opportunity for selection within the species (perhaps due to the fact that our material all comes from one Israeli site (Omer). The propagation of *A.glauca* and selection within this species probably offers more opportunities for selection and will hopefully receive some attention in the coming year.

Selection of indigenous species for the program has had some problems. We are persisting in work with *Boscia*. *Salsola* will probably have to be given a lower priority in the light of the fact that we only have 3 more years of funding. Within these limitations we propose to establish blocks of *Tripteris* and *Sutherlandia*, for characterization studies and to examine the extent of intra-species variation and hence opportunities for selection.

B.4. Analyses of plant material (Israel)

Interspecific and intraspecific differences in digestibility of potential fodder shrubs for sheep were studied. *In vitro* metabolizable energy yields of six individual *Cassia sturtii* shrubs, two *Atriplex nummularia* shrubs and one *Atriplex halimus* shrub were determined. Metabolizable energy yield of the samples was estimated *in vitro* with the Hohenheim Gas method using sheep rumen fluid as the incubating media (Table 1). In this method, the gas produced in anaerobic fermentation of substrate was used to predict the energy value. Rumen liquor and particulate matter were collected before morning feeding from two sheep fed on a roughage diet of mainly poor quality wheat straw and some lucerne hay; the liquor was homogenized, strained and filtered through glass wool. Incubation media was prepared as described by Menke et al. (1979). Samples, each of 200 mg DM, were incubated in triplicate in 100 ml calibrated glass syringes in which 30 ml of the incubation media was added. The glassware was kept at 39°C and flushed with CO_2 before use and the mixture was continually stirred under CO_2 at 39°C. Gas production, as determined by piston movement, was measured over 24 h after correcting for gas production due to rumen fluid alone.

In vitro metabolizable energy yield of the *Cassia sturtii* leaves averaged 4.23 kJ/g DM and of the small branches averaged 4.16 kJ/g DM. Values for *Atriplex nummularia* leaves averaged 4.95 kJ/g DM and for branches averaged 5.19 kJ/g DM, and for *Atriplex halimus* leaves averaged 6.30 kJ/g DM. Thus, differences among species were noted, with *Atriplex* having higher metabolizable energy yields than *Cassia*. This occurred even though the gross energy of *Cassia* was higher than that of *Atriplex*, indicating lower digestibility of *Cassia* than *Atriplex*. Ash content of *Atriplex* was higher than *Cassia* with little difference within species;

crude protein content was higher in *Atriplex* than *Cassia*. In general, metabolizable energy yield is low for all shrubs; however, the shrubs could be an important source of crude protein and could be used as fodder when forage is scare.

Table 1. Composition and *in vitro* metabolizable energy of leaves (L) and small branches (B) of *Cassia sturtii* and *Atriplex nummularia* in Israel. Ash, crude protein (CP) and neutral detergent fiber (NDF) are in percent dry matter (DM). Gross energy (GE) and metabolizable energy (ME) yields are in kJ/g DM.

Plant	I.D.	Ash	CP	NDF	GE	ME
Cassia sturtii	1D 2.3 (L)	9.63	8.54	24.52	18.78	4.04
	1D 2.3 (B)		7.54			4.25
	1D 2.5 (L)		8.73	24.52		4.30
	1D 2.5 (B)		6.78			4.15
	1D 3.3 (L)	8.75	9.22	25.69	19.05	4.32
	1D 3.3 (B)		6.60	55.64		3.82
	4C 3.6 (L)		8.63	25.00		4.32
	4C 3.6 (B)		6.74			4.45
	8D 4.4 (L)	10.78	9.54	23.73	19.06	4.10
	8D 4.4 (B)		7.51			3.97
	8D 6.8 (L)	9.78	9.20	26.38	18.37	4.28
	8D 6.8 (B)		7.51			4.32
Atriplex nummularia	3D 2.5 (L)					5.03
	3D 2.5 (B)		6.20			5.14
	5D 4.3 (L)	28.00	16.43	28.44	13.83	4.86
	5D 4.3 (B)	6.19	7.48	68.75	16.54	5.23
Atriplex halimus	(L)	34.22	16.10	27.32	11.09	6.30
Alfalfa		16.48	17.15	40.58	14.56	7.20
Wheat straw		11.89	5.05	61.85	14.88	5.82

B.5. Analyses of plant material (South Africa)

Data being collected on the differences within and among shrub species will allow the selection of the most promising species and individual shrubs for cultivation and for large-scale clonal propagation. Initial reports on nutritive value of various species and at different sites should

be completed during the coming report year. From 2002/2003 nutritive value assessments will be extended and expanded with respect to *Cassia*, indigenous shrubs and some of the species we are including in the winter rainfall sites.

B.6. Field observations, Omer Fodder Shrub Introduction Site (Israel)

Field trials of at Sede Boqer and at the Omer Introduction site continued. Data was collected on field trips to Omer in February and August 2002. As previously, general observations were made as well as measurements of shrub height and diameter.

In February, plants of *A. nummularia*, *A. canescens* and *Cassia sturtii* had begun to come out of winter dormancy; all bearing young green foliage. *Atriplex* spp. seemed to break dormancy earlier than *Cassia*. *A. nummularia* shrubs bore young shoots with well-developed leaves and most also had flower buds. Vegetative growth on *A. canescens* was less advanced, with short young shoots sprouting. Many shrubs of this species had not recovered from the previous summer's unauthorized overgrazing. They had no young shoots, the main branches look dry and lifeless and only a few bore a small amount of green leaves and buds. *C. sturtii* that had been pruned the previous year showed no young growth. As in the past, *Cassia* shrubs on the edges of blocks bore flowers while the rest did not as yet. The same signs of fungal disease that had been detected in the previous year could be seen.

"Unauthorized" grazing was detected during the August field trip. *A. canescens* appeared to have been preferred by sheep over the other species at the site in both years. All non-woody parts of *A. canescens* shrubs had been browsed and there were no observable signs of recovery. *A. nummularia* had also been grazed, but to a lesser degree than *A. canescens*. These shrubs had begun to recover, with development of new leaves and shoots. The crowns of the highest shrubs were inaccessible and had not been disturbed. Shoots and leaves of almost all *A. nummularia* plants had black spots, indicating fungal disease. "Branches and shoots that had not been grazed bore seed.

Cassia sturtii had also been "grazed". Shrubs bore mature dry pods but not new flowers. Those that had been pruned for cuttings in the spring did not show good recovery with few axillary buds bearing new shoots. Insect damaged pods and seeds of this species were observed at both the Omer site and at Midreshet Sede Boker. Damaged seeds proved not to be viable.

B.7. Rooting of cuttings

Cuttings for rooting experiments were taken at the Omer site as in the previous year. They were transported in plastic bags and processed in the laboratory on the same or the next day. In order to avoid desiccation, cuttings were held in the presence of moist tissue paper in plastic bags in a refrigerator. Half of the cuttings used in the trials were terminal (bearing the shoot tip) and half were sub-terminal. The approximate length of a cutting was 7-11 cm. Leaves were stripped from the basal 3-5 cm of each before insertion into the substrate or nutrient medium. Roots appeared after approximately 4-6 weeks. After the appearance of roots, whether in soil mixtures or in hydroponics, cuttings were transferred to a soil mixture composed of local red clayish soil + sand + peat.

A preliminary experiment was conducted on the rooting of cuttings taken during the spring field trip. Ten cuttings each of *A. nummularia*, *A. canescens* and *Cassia sturtii* from each selected plant were placed in a soil-based substrate (a commercial potting mix produced by Hagarin, Israel, containing tufa, peat, osmocote slow release fertilizer and water absorbing materials) and 10 into aerated Hoagland's solution. All were placed in the greenhouse. Cuttings from both Atriplex species rooted in both solid potting mix and hydroponics, although with differences in the rates of success associated with individual shrubs and among species. None of the *Cassia sturtii* cuttings rooted (Table 2).

A larger trial was conducted with plant material collected during the summer field trip. Twenty terminal and 20 sub-terminal cuttings were taken from selected plants of the three target species. Cuttings were taken from three individual plants of each species. The trial included 5 treatments, as follows:

1.	Pots (vermiculite + peat – 3:1) without Hormoril (commercial rooting powder)
2.	Pots (commercial potting mixture) without Hormoril
3.	Pots (vermiculite + peat) + Hormoril (0.8% IBA+ 5% TBZ)
4.	Pots (commercial potting mixture) + Hormoril (0.8% IBA+ 5% TBZ)
5.	Hydroponics (Hoagland's solution) without Hormoril

Table 2. Rooting rates of cuttings in pots and in hydroponics

Plant no. & species		% rooting in pots	% rooting in hydroponics
1	A. nummularia	42.10	12.00
5	A. canescens	0	0
6	A. nummularia	35.00	50.00
9	C. sturtii	0	0
2	A. canescens	10.00	16.66
12	C. sturtii	0	0
4	A. canescens	15.00	9.00
8	C. sturtii	0	0

Cuttings, prepared as described above were set into solid substrate and hydroponics treatments in 500 ml pots and 9l plastic tubs, respectively. The containers were placed under intermittent mist provided in a 3-minute cycle (1 min spraying and 2 min idle). Nutrient medium in the hydroponics system was changed weekly. Growth of fungi and algae were controlled by periodic spraying with fungicide (Benlate 0.5% solution). The first signs of rooting could be noticed in 4 weeks. After 6-7 weeks data was collected and rooted plantlets were transferred to larger pots containing soil (as described above).

As in the preliminary trial, the most successful rooting was demonstrated by *A. nummularia*, while none of the cuttings of C. *sturtii* rooted. Irrespective of the treatment, better rooting results were attained with terminal shoots than with sub-terminal shoots in both Atriplex species. The best rooting for both *A. nummularia* and *A. canescens* occurred in hydroponics (treatment 5), at average rates of 90 and 23 % respectively. In particulate media rooting was better in media composed of vermiculite and peat (3:1) than in the commercial potting mix, irrespective of whether cuttings were treated with the Hormoril rooting hormone mixture. Rooting was not improved by Hormoril. While rooting was best in hydroponics and the cuttings in hydroponics appeared to be more vigorous, those rooted in particulate mix acclimatized more readily following re-potting (Table 3.). This having been said, 100% of the rooted cuttings from hydroponics and from particulate potting mixes survived acclimatization. There was considerable plant-to-plant

variability within each of the Atriplex species, supporting the thesis that rooting ability may be selected for.

One week after initiation of this trial, most of the *C. sturtii* cuttings dropped foliage and turned brown. The medium turned brown as well, presumably due to phenolic compounds that were exuded from the cuttings. Those few cuttings that survived the initial period, remaining green and swelling at the base of the stem, were transferred to fresh medium. After 4 more weeks no roots were observed (Table 3.).

Table 3. Percentage of rooting of *A. nummularia, A. canescens* and *C. sturtii*

Treatment	% of rooted plants in the treatment									
	1		2		3		4		5	
Plant # &name	terminal	sub-terminal	term	sub-term	term	sub-term	term	sub-term	term	sub-term
#1 *A. nummularia*	55	25	45	15	60	20	45	5	95	10
#3 *A. nummularia*	60	30	35	0	55	20	15	5	85	0
#6 *A. nummularia*	70	25	50	20	75	35	40	10	90	0
A. numm. avg.	**62**	**27**	**43**	**12**	**63**	**25**	**33**	**7**	**90**	**3**
#2 *A. canescens*	10	0	5	0	10	0	0	0	35	5
#5 *A. canescens*	0	0	0	0	5	0	0	0	25	0
#4 *A. canescens*	15	0	0	5	5	0	0	0	10	0
A. canses. avg.	**4**	**0**	**2**	**2**	**7**	**0**	**0**	**0**	**23**	**2**
#8 *Cassia sturtii*	0	0	0	0	0	0	0	0	0	0
#9 *Cassia sturtii*	0	0	0	0	0	0	0	0	0	0
#12 *Cassia sturtii*	0	0	0	0	0	0	0	0	0	0
C. sturtii avg.	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

B.8. *In vitro* studies

Work continued on the *in vitro* approaches described in our previous reports, conventional micropropagation, proliferation of callus and regeneration, and somatic embryogenesis.

Micropropagation

A. nummularia

Variations of MS basal medium were used in our earlier micropropagation work. In recent trials with *A. nummularia* (Omer shrub 3) aimed at improving micropropagation, full and half strength (50% macro- and micro-nutrients) WPM, MS, SH and BPR were tested. Each medium was supplemented with 30g/l sucrose and gelled with 6g/l agar. Shoot segments, 9-15 mm in length, produced *in vitro* on hormone-free MS medium, were used. Expanded leaves and apical buds were removed. Each treatment was replicated 15 times.

Shoot height, the number of buds and leaves per explant and the number plantlets that rooted in each medium were measured four weeks after initiation of the trial. According to all parameters tested, full strength MS promoted the best development. Whereas the average shoot height ranged between 19.27-24.87mm in the other treatments, it was 52mm in full strength MS. Similarly the number of buds and leaves produced and the rate of rooting were 2-3 times greater in MS than in the other media tested (Table 4). Shoots and plantlets in this treatment appeared to be healthy and vigorous; all bore green and broad leaves with thick stems and numerous roots.

Table 4. Responses of *A. nummularia* cultures to culture media (data represents the averages of 15 replicates)

Treatment	Medium	Height, mm	# of buds	# of leaves	Roots, %
1	MS 100%	52.00	9.00	9.60	100.00
2	MS 50%	24.87	4.60	4.80	33.33
3	WPM 100%	19.27	3.33	4.13	26.67
4	WPM 50%	20.20	2.93	4.27	33.33
5	BPR 100%	25.69	4.54	5.69	46.15
6	BPR 50%	22.86	3.21	3.93	21.43
7	SH 100%	19.67	3.58	4.08	41.67
8	SH 50%	19.86	3.71	5.14	57.14

Attempts were made to accelerate shoot proliferation in liquid culture. The same source of shoots as described above was used. Explants were 1-1.5 cm long, bearing at least 2 axillary buds (nodes). Expanded leaves were removed. In a series of trials, explants were placed in

liquid MS medium containing no hormones, IAA or flurprimidol. None of the treatments gave satisfactory results. We will continue to pursue this direction.

Trials were conducted on shoot propagation from adventitious buds. The best medium for initiation of shoot proliferation was full-strength hormone-free MS. Microcuttings, each having 2-3 buds, were multiplied on media containing a range of concentrations of IAA or IAA and BA. Extension, branching and rooting occurred at different rates at each IAA concentration. The greatest number of buds (greatest multiplication potential) produced, greatest height attained and highest percentage of plantlets that rooted occurred on 5 mg/ IAA (table 5).

Table 5. Effects of IAA and BA on proliferation and rooting of *A. nummularia* microshoot cuttings.

IAA, mg/l	BA, mg/l	no. shoots	no. buds	plantlet height, mm	rooting, %
0.5	0	1.29	5.76	39.82	23.00
5.0	0	1.00	8.00	56.43	81.25
10.0	0	1.00	5.21	26.35	64.82
0.5	0.2	1.37	2.5	17.25	37.50
5.0	0.2	1.00	1.75	12.50	25.00

Acclimatization: In a preliminary attempt to acclimatize or "harden off" plantlets produced *in vitro* on full strength hormone free MS medium, the jelled substrate was washed from the roots with running tap water and the plantlets were placed into a mixture of vermiculite and peat (2:1). Plants were placed in a mist chamber inside a greenhouse and gradually removed from the mist chamber after one week. They were exposed to dryer conditions for one hour on the first day, for two hours on the second day, etc. until at the end of the second week plants were completely moved from the mist chamber to the standard greenhouse environment. Five weeks 37 of 39 plants had survived; a survival rate of 95%.

A subsequent trial was conducted, in which the influences on acclimatization of substrates and mother plant origin were tested. Plantlets derived in culture from Omer shrubs 3 and 6 were used. 10 plantlets of each were planted out on a vermiculite-peat mixture (as in the previous trial) or on a commercial potting mix. After 34 days, significantly fewer plants of each origin had

survived in the commercial potting mixture than in the vermiculite-peat mixture (Table 6), perhaps because of the higher concentration of nutrients in the commercial mix. The results support continued use of the vermiculite-peat mixture for acclimatization of *A. nummularia*.

Table 6. Survival of ramets produced from two *A. nummularia* shrubs on different substrates

Plant no	substrate	% survival
3	Vermiculite+Peat	60
6	Vermiculite+Peat	70
3	commercial mixture	40
6	commercial mixture	30

A. canescens

Mei et al (1997) reported that the most suitable medium for initiation and growth of *A. canescens* was half strength MS medium supplemented with 0.1 mg/l of IAA and 0.5 mg/l of BA plus 450 mg/l of NH_4Cl. A trial was initiated using this medium. Cuttings were taken from greenhouse-grown plants and disinfested for 3-4 min in a 0.5% benlate solution followed by 10 min in 20% commercial bleach solution and washed 4 times in sterile distilled water. Stem segments, 1-1.5 cm long and bearing 1-2 buds, were excised aseptically. 108 test tubes were inoculated with explants.

After four weeks of culture, only two explants had developed small shoots without roots. These were transferred to fresh medium of identical composition, but consequently died. All other explants turned light brown in color. Similar results were obtained when *A. canescens* was grown in full strength MS medium without growth regulators.

Efforts to initiate cultures of this species and to develop a micropropagation will continue.

Cassia sturtii

Cultures initiated from shrubs of *C. sturtii* growing in the field always became contaminated. Therefore, explants were prepared from seedlings. Seeds were germinated aseptically *in vitro*, shoots were excised for micropropagation trials and cotyledons were used in efforts to induce indirect organogenesis and somatic embryogenesis.

Explants exuded phenolic compounds into the medium. Foliage yellowed, indicating the evolution of ethylene. Activated charcoal and the antioxidants citric and ascorbic acid were incorporating into the medium in an effort to alleviate these effects. Shoots with apical and axillary buds were cultured on Woody Plant Medium (Lloyd and McCown, 1981) supplemented with 2, 3 or 4 g/l activated charcoal and 75mg/l each of citric and ascorbic acids. 25 replicates were prepared per treatment. Data was collected after 1 month of culture. Activated charcoal reduced leaf yellowing and abscission. The lowest concentration of activated charcoal tested, 2 g/l, resulted in production of the longest shoots, the most buds, more green leaves and fewer yellow leaves per cutting and more rooted plantlets (Table 7). In general, plantlets that developed roots were the longest and had fewest yellow leaves. Explants derived from basal tissue rooted better than apical explants

Table 7. Responses of *C. sturtii* cultures to activated charcoal (AC) and antioxidants. The data represents averages of all treatments.

AC, g/l	Shoot length, mm	No. of buds	No. of green leaves per test tube	No. of yellow leaves per test tube	% of rooted plants
2	32.54	4.69	4	1.38	77.00
3*	22.15	3	1	2	30.76
4*	23.46	2.92	2.33	2	38.46

In another trial aimed to alleviate the ethylene problem, silver thiosulfate (STS), an ethylene inhibitor, was incorporated into WPM at 0.1–0.4 M. Data was collected after one month of culture (Table 8). In general, culture quality was enhanced more by activated charcoal than STS The highest STS concentration (0.4 M) caused significant reduction in the number of shoot-forming cultures and a marked increase in the number of dead cultures. Explants that did produce shoots in the presence of STS remained short (<10mm), leaves were small and dry along their edges, and many abscised. In contrast, in the presence of 3 mg/L AC and without STS, all cultures formed shoots and approximately 40% of the cultures rooted (Table 8).

Table 8. Responses of *C. sturtii* to silver thiosulfate (STS)

Treatment	Concentration		Shoot-forming explants, %	Root-forming explants, %	green leaves per culture, avg.	yellow & dry leaves per culture, avg.	dead plants per treatment, %
	STS, M	AC, g/L					
1	0.1	-	57.14	00.00	0.64	1.64	21.42
2	0.2	-	50.00	00.00	0.64	2.71	42.85
3	0.3	-	64.28	00.00	1.36	2.00	7.14
4	0.4	-	25.00	00.00	0.25	0.75	75.00
5	-	-	53.33	00.00	1.27	0.73	40.00
6	-	3	100	42.86	3.64	0.71	00.00

Callus proliferation and regeneration: Immature *C. sturtii* embryos were cultured on WPM medium with different concentrations of 2,4-D (0; 0.1; 1.0; 3 mg/l). The best auxin concentration for callus induction was determined to be 3 mg/l 2,4-D when cultures were cultivated in darkness. The callus that formed was hard, compact, light green, with small spherical cells on its surface and highly oxidized on the lower surface in contact with the media. Callus cultures were transferred to media supplemented with 2,4-D and cytokinin (BA and Kin) for proliferation and then to media containing 1 μM TDZ with or without 1 mg/l 2,4-D. On the surface of calli cultivated on WPM with TDZ and lacking 2,4-D, bud formation occurred, followed by shoot development during a 6-week culture period.

Callus and regeneration also occurred on cotyledons from seeds germinated *in vitro*. A variety of hormone combinations (1 mg/l 2,4-D, IAA or IBA combined with 0.1 or 1 mg/l kinetin) and casein hydrolysate were added to the initiation media. Antioxidants (ascorbic acid, citric acid and silver thiosulfate) were also added to the media in order to decrease oxidation. Callus developed from the basal part of 95 % of the cotyledons on media supplemented with 1 mg/l 2,4-D and 1 mg/l kinetin. Friable callus formed on the basal parts, edges and surfaces in contact with the media of all cotyledons on this medium supplemented with casein hydrolysate. In the treatments where IAA or IBA was added to media, shoots arose from the basal parts of a few of the cotyledons.

Haloxylon aphyllum

Growth regulators: An effort was made to induce indirect somatic embryogenesis of *H. aphyllum* by culturing callus on media containing a number of growth regulators and/or media additives. Callus induced from cotyledons and hypocotyls was maintained on BRP media containing 2 mg/l 2,4-D and 1 mg/l Kin, or 1 mg/l 2,4-D and 0,1 mg/l Kin, or only 1 mg/l 2,4-D. The highest growth value (GV[1]), 21.13 ± 1.50, was attained on callus initiated from hypocotyls maintained on BRP with 1 mg/l 2,4-D (figure 1). Green, translucent, very friable callus formed with spherical cells on its surface. Deep dark green centers were observed in the callus after 5 months of cultivation.

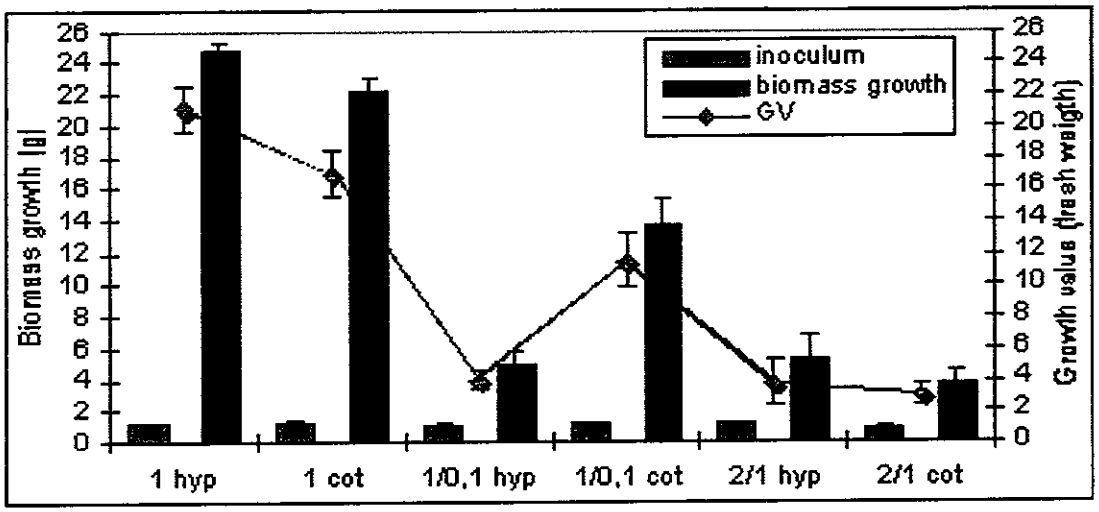

Figure 1. Growth evaluation of callus cultures initiated on different media.
(**1 hyp, 1 cot** = cultures initiated from hypocotyls or cotyledons on BRP medium containing 1 mg/l 2,4-D;
1/0,1 hyp, 1/0,1 cot = cultures initiated from hypocotyls or cotyledons on BRP medium containing 1 mg/l 2,4-D and 0,1 mg/l BA;
2/1 hyp, 2/1 cot = cultures initiated from hypocotyls or cotyledons on BRP medium containing 2 mg/l 2,4-D and 1 mg/l BA)

Abscisic acid: Calli treated with 40 or 20 μM Abscisic acid (ABA) (following work of Nishiwaki et all, 2000), under light or in darkness, browned by the end of 4 weeks of

[1] GV = the change in mass from time 0 to the end of the culture period, calculated as the ratio of fresh weight at time "m_t" (after 4 weeks of cultivation) to fresh weight of the inoculum „m_0" (time 0) (GV = $(m_t-m_0)/m_0$) (Collin a Edwards, 1994).

cultivation. The highest GV, measured in the treatment with 40 µM of ABA in darkness, was only 0.54 ± 0.09 (figure 2). Cells on the callus surface in all treatments were white and elongated. The calli treated with 20 µM ABA gave rise to translucent globular and slightly elongated protuberances. These calli turned brown and died following transfer to medium containing an auxin and cytokinin.

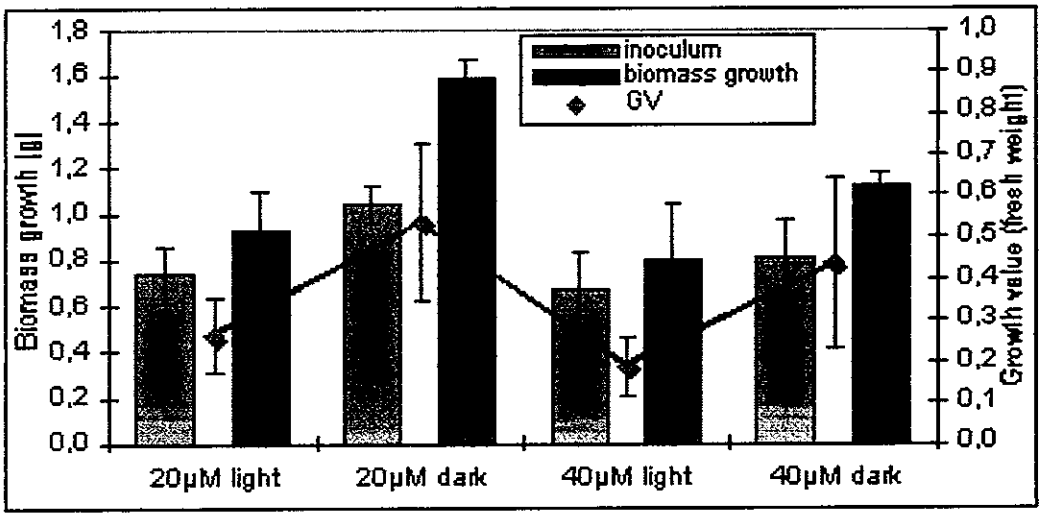

Figure 2. Effect of Abscisic acid on biomass growth of callus cultures.

Activated charcoal and casein hydrolysate: A series of treatments in which casein enzymatic hydrolysate (0.5 g/l), L-Glutamine (0.5 g/l), and a range of concentrations of activated charcoal (0 – 5 g/l) were added to the medium and the saccharose concentration was reduced (0.2 %), gave less desirable results, with the highest GV only 0.16 ± 0.15 in the control treatment under light which lacked activated charcoal. In contrast to the ABA treatments, however, the majority of cultures in this series remained green.

Cytokinins: In an experiment where calli derived from different primary explants and different media were cultured on basal BRP media containing a range of concentrations (0.5, 1.0 and 2.0 mg/l) of the cytokinins Kin, BA and 2iP, significant differences were observed. In all treatments in which the inoculum was callus derived from cotyledons that had been cultured on BRP medium with 2 mg/l 2,4-D and 1 mg/l Kin, there was very little proliferation of callus. The cultures remained green but there was no indication of the occurrence of somatic embryogenesis. On the other hand, cultures of various other origins transferred to the same cytokinin-containing

media turned brown and did not proliferate. There were no significant differences between the cytokinin treatments.

Establishment of suspension culture: Calli produced on BRP media containing 2 mg/l 2,4-D and 1 mg/l Kin, or 1 mg/l 2,4-D and 0,1 mg/l Kin, or only 1 mg/l 2,4-D (maintenance media) were transferred to liquid media of the same composition. The calli disintegrated into individual cells and small clumps. The clumps remained green and increased in size. The individual cells and very small cell aggregates turned brown during the first days after transfer but recovered, became green and proliferated after a subsequent transfer to fresh liquid medium. This indicates that the potential exists to develop a system for *Haloxylon* on liquid media, which is a key to labor-saving automation.

Macrosalt stress: In one experiment, callus cultures were exposed to macrosalt stress (following the report of Choi et al, 1998) by increasing the concentration of KNO_3 in BRP basal media or by adding a high concentration of NH_4NO_3. This was conducted on semisolid as well as liquid media. In all treatments, the basal media were supplemented with 1 mg/l $CaCl_2$. Calli on semisolid media in which either the concentration of KNO_3 or of NH_4NO_3 was elevated (53.41 mM and 31.23 mM respectively) revealed higher rates of proliferation (GVs of 2.05 and 1.87 respectively) than did calli on the control (basal BRP medium) and other treatments (figure 3). The callus that formed on media containing elevated N concentrations became white to brownish. The cells that formed on 31.23 mM NH_4NO_3 were small and spherical, while those on 53.41 mM KNO_3 were elongated. Cultures on liquid medium turned brown within two weeks and did not recover after subculture to fresh high N liquid media.

Flurprimidol: The effect of flurprimidol (0 to 9 mg/l) was tested on callus cultures. In media containing 1, 3, 5 and 7 mg/l flurprimidol, part of the original callus appeared to deteriorate, becoming brownish, but deep green, spherical, compact, meristem-like cell clumps, approximately 1 - 3 mm in diameter, formed from within this tissue. The highest GV (1.87 ± 0.63) was measured at the lowest flurprimidol concentration tested (figure 54). The deep green meristematic clumps were transferred from the flurprimidol treatments to media containing

GA_3 or GA_3 in combination with BA, in order to induce regeneration. The clumps fragmented on the GA media into smaller masses, becoming green-brown in color. Growth was significantly greater in the treatment containing both GA and BA (GV varied from 3.20 to 3.53 on cultures treated previously with 1, 3 and 5 mg/l flurprimidol) but regeneration was not observed (figure 5).

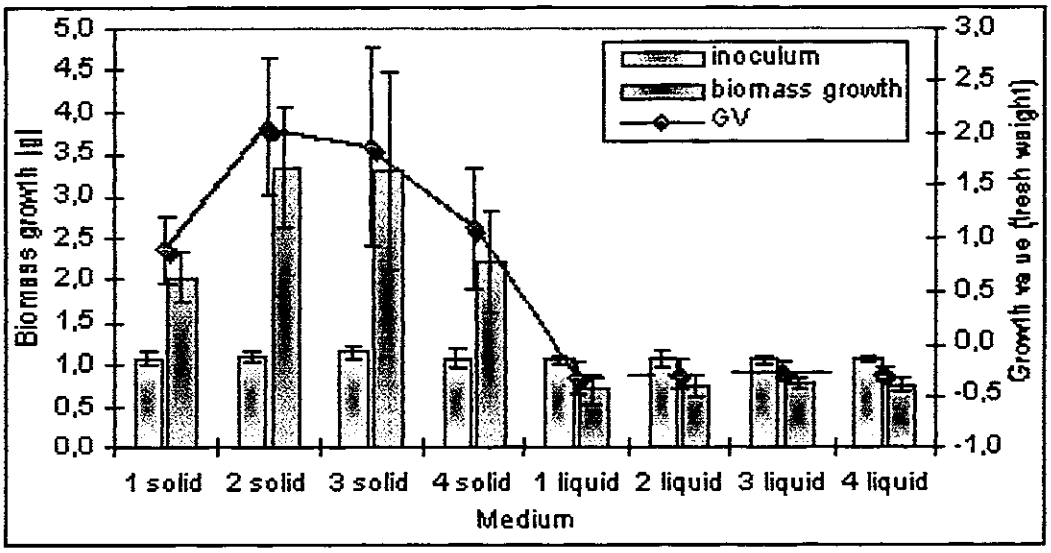

Figure 3. Effect of macrosalt stress on biomass growth of cultures cultivated on solid medium and in liquid medium.
Medium 1 = BRP medium + 1 mg/l $CaCl_2$;
Medium 2 = BRP medium + 1 mg/l $CaCl_2$ + 53.41 mM KNO_3;
Medium 3 = BRP medium + 1 mg/l $CaCl_2$ + 31.23 mM NH_4NO_3;
Medium 4 = BRP medium + 1 mg/l $CaCl_2$ + 62,47 mM NH_4NO_3

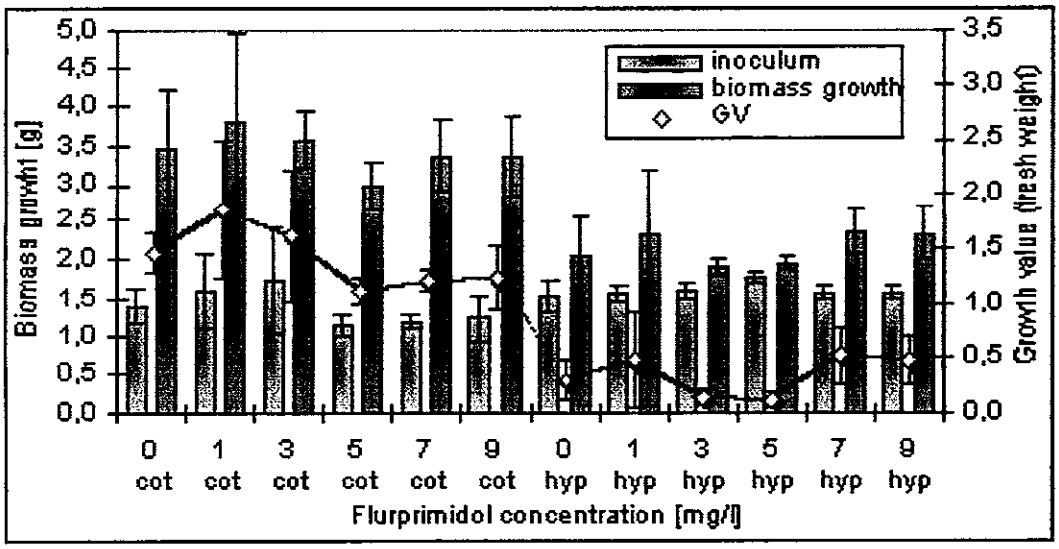

Figure 4. Effect of flurprimidol on biomass growth of callus cultures.
(**cot** = cultures initiated from cotyledons; **hyp** = cultures initiated from hypocotyls).

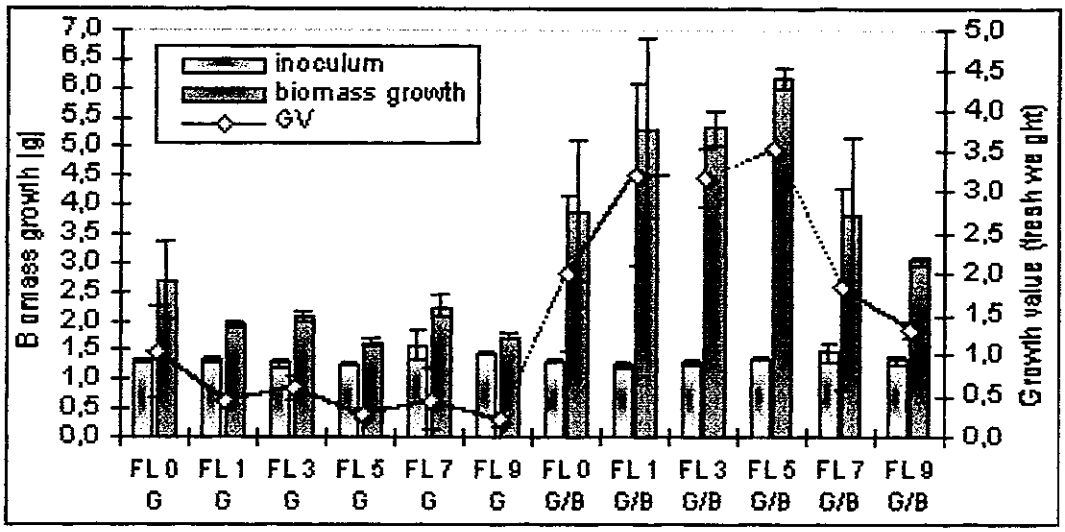

Figure 5. Effect of gibberellic acid on cultures treated previously with flurprimidol.
(**FL 0 – 9** = Flurprimidol concentration in previous treatment; **G** = medium containing 1 mg/l GA; **G/B** = medium containing 1 mg/l GA and 1 mg/l BA)

Thidiazuron: Thidiazuron (TDZ) has been reported to be an effective cytokinin for the induction of both direct and indirect somatic embryogenesis (Rugkhla and Jones, 1998; Tivarekar and Eapen, 2001; Gallo-Meagher and Green, 2002). In an experiment in which the effect of TDZ (1, 2 and 3 μM) was tested on callus, significantly higher proliferation was observed than had been in previous experiments. The highest GV (5.2 ± 1.82) was induced at the lowest concentration of TDZ (figure 6). However the calli became hard, sage-green in color, and oxidized on the lower surface, causing the media to become brown. Cells on the upper callus surface were small and spherical.

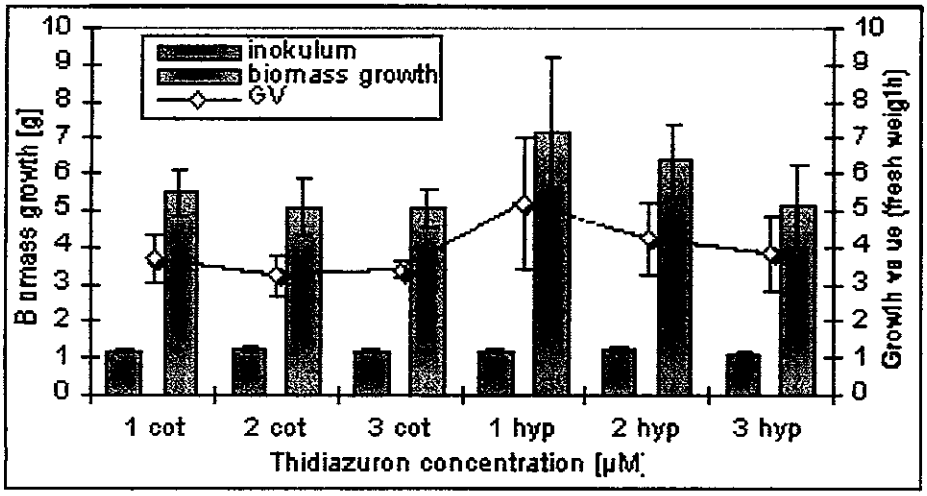

Figure 6. Effect of thidiazuron on biomass growth of callus cultures.
(**cot** = callus cultures had been initiated from cotyledons; **hyp** = callus cultures had been initiated from hypocotyls)

C) Scientific Impact of Collaboration

The collaboration has continued during this period as during the first year of the program. Each of the collaborating scientists has been active in his respective area of responsibility, in both research and training; each has collaborated with the others, with been frequent exchange of data and thoughts. Work on selection has been pursued by all members of the team; Birnbaum in Israel and Rethman in South Africa have focused on agronomic aspects and clonal propagation while Degen and Van Niekerk have worked in Israel and South Africa, respectively, on nutritional evaluation of the shrubs included in the plan of work (Degen) and of additional promising fodder species (Van Niekerk). Graduate students and postdoctoral fellows have been working on aspects of the program at both Universities, with input of the BIDR collaborators in the work of the UP students (see section II, D, below).

D) Description of Project Impact

It is too early in the project to claim impact through implementation of results. However, the frequent communication between the collaborating scientists has been valuable, as has input of the Israeli collaborators to the training of UP graduate students and technical staff. There has been positive and valuable development of relations with the Bushmen (represented by agricultural extension agent Mr. Desmond Smith) and Afrikaner ranchers (individuals cited in section IID, below) involved in animal husbandry and fodder shrub trials in the Northern Cape, which will be vital to implementation of the results of these studies in the future.

E) Strengthening of Developing Country Institutions

The training of a technician and graduate student in Degen's laboratory at the BIDR last year have led to preparation of appropriate equipment and implementation of the method used in Degen's lab for evaluations of the energetic value of fodder shrubs at UP. Further training, of a second technician and another graduate student from UP, in the area of clonal propagation and tissue culture, will be carried out in Birnbaum's lab at the BIDR in the spring of 2003.

Fodder shrub evaluation sites have been established at several locales (see section IB, above) from seed transferred from Israel to South Africa, serving as the basis of large parts of this program.

F) Future Work

The work is, in general, following the schedule presented in the proposal.

a. Shrub selection has continued in Israel and in South Africa. Evaluations have continued of the fodder plant populations at the Omer site and on the grounds of the BIDR in Israel, as well as at introduction sites in the Northern Cape. Studies of individual plant productivity, palatability and energetic value have been made in both countries. The behavior of individual shrubs *in vitro* has been studied in Israel. These efforts will continue into the coming year.

b. New species, selected in South Africa (including *Boscia, Salsola, Rhus Ziziphus, Opuntia, Agave, Harpagophytum, Sutherlandia* and *Tripteris.*), have been added to the list for study and consideration. These have been planted at various trial sites in South Africa and will continued to be evaluated.

c. The system for estimating metabolizable energy yield of forage was applied at BIDR to several shrubs of *Cassia sturtii, Atriplex nummularia* and *Atriplex halimus*. Its use will be expanded to evaluate all of the shrubs that have been targeted in the program at Omer and at Sede Boqer. Parallel studies on target species will be carried out in South Africa.

d. A number of agrotechnologies, including water harvesting, soil disturbance by tractor and mechanical ripper, the use of soil ameliorants, alley cropping, and application of mulches have and will continue to be tested at trial sites in South Africa.

e. Development of clonal propagation systems by cuttings and tissue culture has and will continue at the BIDR. A postdoctoral fellow will join the BIDR tissue culture group in the spring of 2003 to continue the work there. The greatest attention will be paid to A. canescens

and *C. sturtii*, both of which have been relatively recalcitrant. A technician and graduate student from UP will receive training in tissue culture at the BIDR in the spring of 2003, after which efforts in this area will be intensified at UP.

Section II

A) Managerial Issues

Concerns for personal security have resulted in delays in visits of the UP collaborators and personnel to Israel. They are now scheduled for the spring of 2003 (see section D, below).

B) Budget

none

C) Special Concerns

none

D) Collaboration, Travel, Training and Publications

Collaboration

In addition to the excellent collaboration between the scientific collaborators, productive collaboration has been established with leaders in the farming communities where field trials have been established in South Africa: with Mr. Kolie van Wyk at Mier, Mr. Gert Olivier at Hotazel, Mr. Albert van Niekerk at Kenhardt and Mr. Gert Olivier at Pofadder. The cooperation of these individuals has been essential in conducting field trials and will be of great value at a later stage in bringing the results to these communities for implementation.

Training

The group of M.Sc. students at UP has been enlarged, to include Mr. Francois Sparks, Mr. Lindeque Du Toit, Ms. Annelize Van der Baar and Mr. Pieter Vermaak, who are working primarily with animal science aspects, while Mr. Danie Venter and Ms. Trove Wilcock have

concentrated on plant aspects. Ms. Christa Havenga has joined the team as a PhD candidate. With Ms. Havenga and Ms. Wilcock coming into the UP team, inputs, particularly in the area of vegetative propagation, using both cuttings and tissue culture, are being increased and the BGU expertise will be have greater impact. Four undergraduate students have also been involved at UP in germination studies conducted as part of their training. This has entailed a wide range of treatments applied to *Boscia*, *Tripteris* and *Sutherlandia*

Graduate students have been involved in the fodder shrub trials, as follows:

- Sparks (M.Sc. Agric.) has studied the quality of edible plant material. The second draft of his thesis has been completed and he is almost ready for examination.
- Du Toit (M.Sc. Agric.) has concentrated on the energy supplementation of oldman saltbush, *A. nummularia*. He has finished with the collection of data and lab analyses and has begun to prepare the first draft of his thesis.
- van der Baar (M.Sc. Agric.) had training in Degen's lab at BIDR last year and has worked on analysis of the nutritive value of drought tolerant species. He has finished with the collection of data and lab analyses and has begun to prepare the first draft of his thesis.
- Vermaak (M.Sc. Agric.) has addressed the degradability and fermentation of components of fodder shrubs. He is currently conducting the lab analyses.
- Wilcock is involved in trials of Hatfield Elite Plant Progeny (plants grown from seed harvested from shrubs selected for palatability in earlier trials. He has also produced the first batch of *Cassia* seedlings, to be planted in December 2002, and is producing more seedlings for an autumn planting at Hatfield. These will be used in studies of nutritive value and forage characterization.
- Venter is conducting a study of the productivity and relative palatability of fodder species and will establish plantings of new species of interest at both Hatfield and field sites for characterization studies.
- Wilcock is studying the development of *Cassia* and *A. nummularia* seedlings, including problems of herbivory, planted in riplines, with and without protection.
- Havenga (Ph.D. student) is evaluating such interventions as water-harvesting from bare areas, stone mulches to reduce evaporative losses, water absorbent polymers to improve

water holding capacity and use of thorn branches to protect plants against herbivory, using two tree species, *Rhus* and *Ziziphus* planted on bare areas.

- Andrei Korchagin, M.Sc. student from Kazakhstan, who has been working at the BIDR on Atriplex and Cassia propagation by rooting cuttings and by micropropagation, is writing up the results of his work and will submit his thesis in the spring.
- Lenka Langhansova, Ph.D. from the Czech Republic, completed postdoctoral studies in the tissue culture laboratory of the BIDR in June 2002. Dr. Langhansova collaborated with Birnbaum and Korchagin on in vitro studies toward micropropagation and with Birnbaum on embryo and callus culture and regeneration.
- A new postdoctoral fellow will join the BIDR tissue culture lab in the spring of 2003 to continue tissue culture studies.

Travel

- Rethman made a presentation to the UNCCD meeting in Cape Town, while the "team" made a series of presentations at the SASAS/GSSA congress in North West Province and 2 – 3 papers have been accepted for the International Rangeland Congress to be held in Durban in July 2003.
- Wilcock and Havenga attended the Arid Zone Forum, held this year in Middleburg, Eastern Cape.
- Venter participated in a vegetation workshop in Western Australia.
- A planned visit by the collaborating scientists, Rethman and Van Niekerk, delayed from this reporting period due to security concerns, is now scheduled for spring 2003. Their goal, in addition to conferring with the Israeli collaborators, is to learn as much as possible about water harvesting in Israel and about livestock production systems in the Negev.
- Graduate student Ms. Christa Havenga and technician Ms. Annemarie Liebenberg, who also were originally scheduled to come to Israel earlier, will also come to the BIDR in the spring for one month of training and hands-on experience in tissue culture.

E) Request for American Embassy Tel Aviv or A.I.D. Actions

none