

Distribution of thiophenes in *Tagetes mendocina* Phil. and *Tagetes argentina* Cabrera

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ABSTRACT

The distribution of four thiophenes, a-terthiophene (α -T), 5-(but-3-en-1-ynyl)-2,2'-bithiophene (BBT), 5-(4-hidroxy-1-butynyl)-2,2'-bithiophene (BBTOH) and 5-(4-acetoxi-1-butynyl)-2,2'-bithiophene (BBTOAc) was studied during the first 15th weeks of the ontogenetic cycle in two wild species of *Tagetes*. The four mentioned thiophenes were detected in roots and shoots of *T. mendocina* and *T. argentina* in variable concentrations along the experimental period. The roots of both species were the site of the major accumulation. BBT showed the highest concentration in the whole plant. In *T. argentina* the high content was found just before flowering while in *T. mendocina*, the total thiophene concentration reached the maximum value in the 8th week, seven weeks before flowering.

Key words: thiophenes, *Tagetes*, secondary metabolites, distribution

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RESUMEN

Se estudió la distribución de 4 tiofenos, a-tertiofeno (α -T), 5-(but-3-en-1-inil)-2,2'-bitiofeno (BBT), 5-(4-hidroxi-1-butinil)-2,2'-bitiofeno (BBTOH) y 5-(4-acetoxi-1-butinil)-2,2'-bitiofeno (BBTOAc) durante 15 semanas del ciclo ontogénico de 2 especies nativas de *Tagetes*. Los 4 tiofenos mencionados se detectaron en raíces y tallos de *Tagetes mendocina* y *Tagetes argentina* en concentraciones variables a lo largo del período experimental. Las raíces de ambas especies fueron el sitio de mayor acumulación. En la planta entera, BBT fue el compuesto que alcanzó mayor concentración. En *T. argentina* el contenido más elevado fue detectado justo en el momento previo a la floración, mientras que en *T. mendocina*, la concentración total alcanzó su máximo valor en la octava semana, siete semanas antes de la floración.

Palabras clave: tiofenos, *Tagetes*, metabolitos secundarios, BBT, BBTOAc, BBTOH, α -T

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INTRODUCTION

The occurrence of thiophenes in many tribes of the *Asteraceae* is well known. They represent a group of natural products with a wide distribution. To date, they have been found in several species of *Tagetes*, *Dyssodia* and *Bidens*. The knowledge about these phototoxic compounds has grown considerably (Bohlmann *et al.*, 1973; Bohlmann and Zdero, 1985). *T. patula* is the most studied plant of the genus (Norton *et al.*, 1985; Tosi *et al.*, 1988; Downum and Towers, 1983; Ketel, D., 1987). It was reported that both BBT and α -T were phototoxic to bacteria and yeast in UV-A, but they were not lethal in the dark (Chan *et al.*, 1975). The potent nematocidal properties of α -T has been demonstrated to be enhanced by near UV light (Bakker *et al.*, 1979; Gommers, F., 1972; Arnason *et al.*, 1981a). The effects of these compounds have been demonstrated with algae and insects (Arnason *et al.*, 1981b; Hudson *et al.*, 1986a).

It was found that these secondary metabolites occurred in different concentration in all plant tissues (Ketel and Breteler, 1988). The distribution of thiophenes within the plant during the growth cycle has been studied in seedlings and hydroponically grown plants (Tosi *et al.*, 1988; Downum and Towers, 1983; Sutfeld, R., 1982). Up to now, the distribution of thiophenes along the ontogenetic cycle has been studied only in *T. patula* (Tosi *et al.*, 1988). The accumulation in different organs of *T. patula* seedlings grown under various conditions has been studied (Sutfeld, R., 1982).

Our study was focused in two native species, *T. mendocina* and *T. argentina*. We analyzed the distribution and accumulation of the main four thiophenes in roots and shoots of *T. mendocina* and *T. argentina* during 15 weeks of culture of glasshouse grown plants in order to establish the possible relationship between the developmental state and the variation of the metabolites in the whole plants

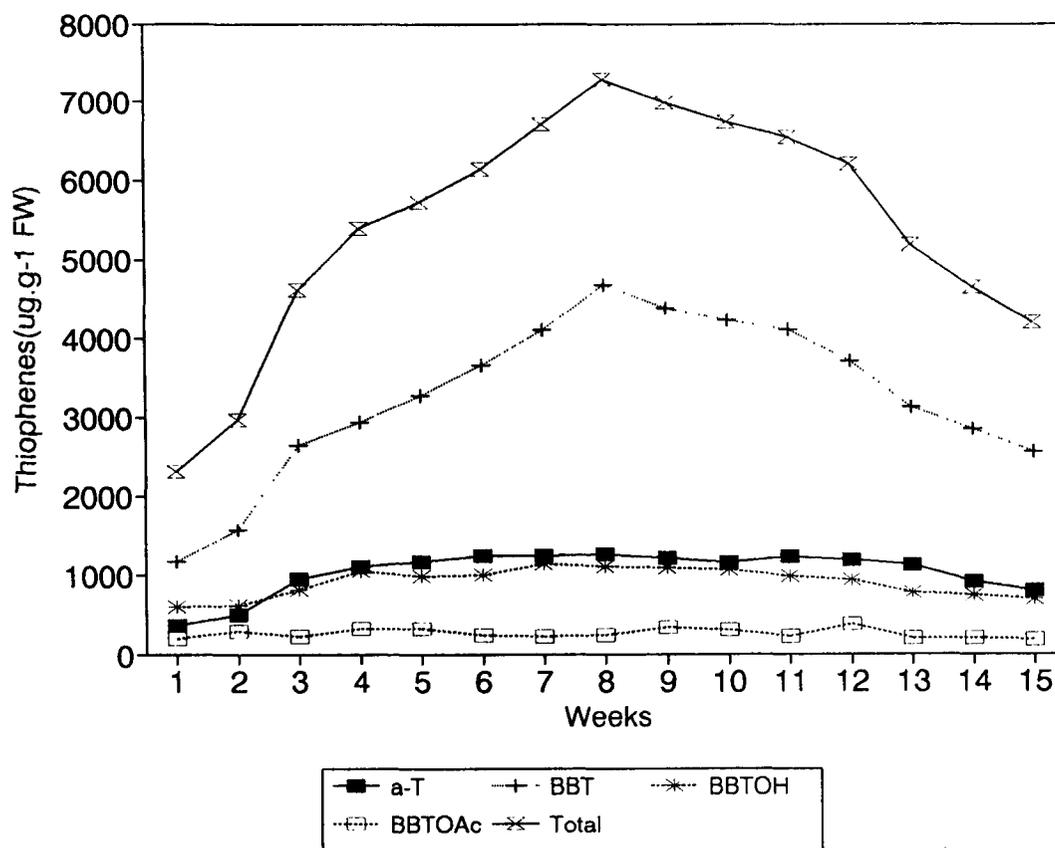


Fig. 1. Thiophene content in shoots of *T. mendocina* along 15th weeks of culture ($\mu\text{g/g FW}$)

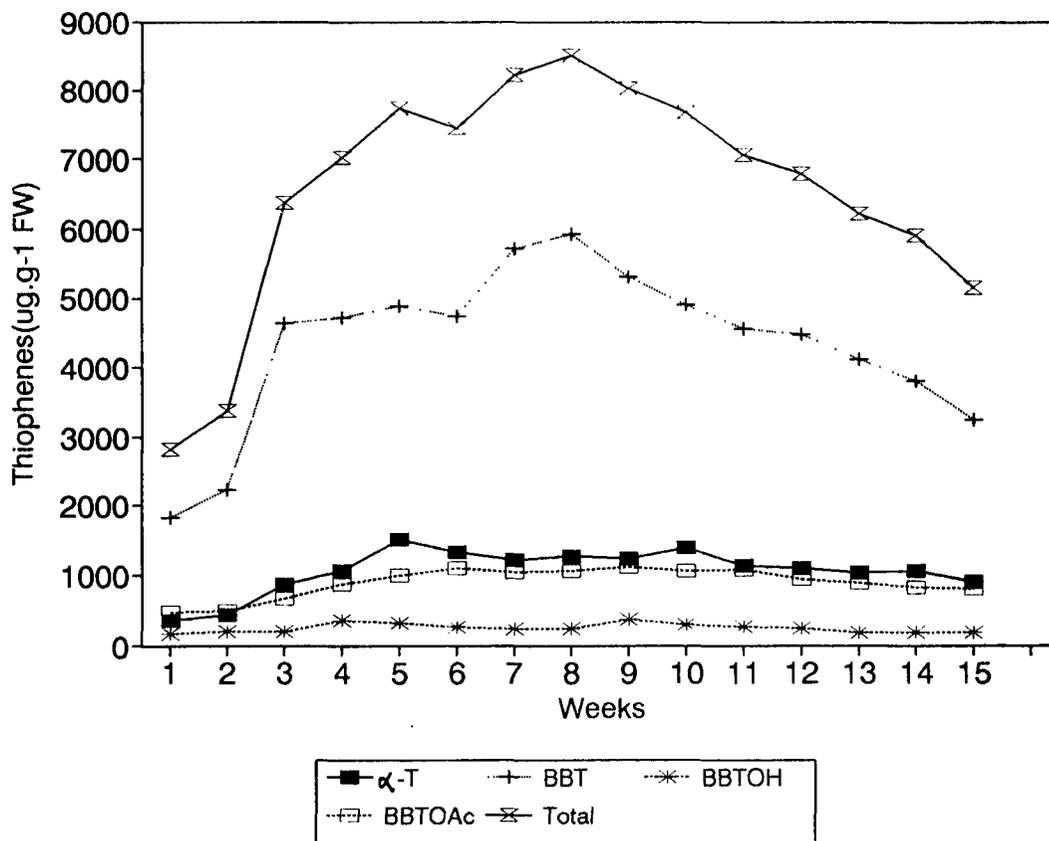


Fig. 2. Thiophene content in roots of *T. mendocina* along 15th weeks of culture (µg/g FW).

MATERIALS AND METHODS

Plant material

Seeds of *T. mendocina* and *T. argentina* were collected in San Rafael (Mendoza) in autumn, 1991. The glasshouse plants were grown under natural light conditions in 1L pots, filled with perlite and watered with Hoagland (Hoagland and Arnon, 1938) solution every second day. Plants were harvested each week, roots and shoots were weighed separately, extracted and kept at -20°C until analysis.

Extraction and determination of thiophenes

The distribution of thiophenes was checked qualitatively and quantitatively along fifteen weeks. During this experimental period, *T. mendocina* maintained the vegetative state until the end of the experiment, flowering in the 15th week. *T. argentina* flowered in the 12th week, beginning with the stage of fruiting towards the 14th week.

Thiophene extraction was carried out under dim room light, according to Norton *et al.* (1985). Roots and shoots were separated, cut in pieces and a representative sample of 500-1000 mg was separated and extracted using methanol at room temperature, following the procedure of Norton (1985). The residue was resuspended in HPLC grade methanol and stored at -20°C for TLC and HPLC analysis. For TLC, samples were spotted on silica gel plates (GF 254) with a fluorescent indicator and developed in methanol. Thiophene bands were observed under UV light (254 nm). Standards of BBT, α -T, BBTOH and BBTOAc were used for comparison. Only qualitative analysis was made on TLC.

For HPLC, qualitative and quantitative analysis were made. Isocratic separations were performed on a Spherisorb SS ODS-2 column (125 x 4mm), using methanol:water (85:15) (Merck) as eluent at a flow rate of $1\text{ ml}\cdot\text{min}^{-1}$. The eluant was monitored at 333 nm with a Hewlett Packard 1050 variable wave-

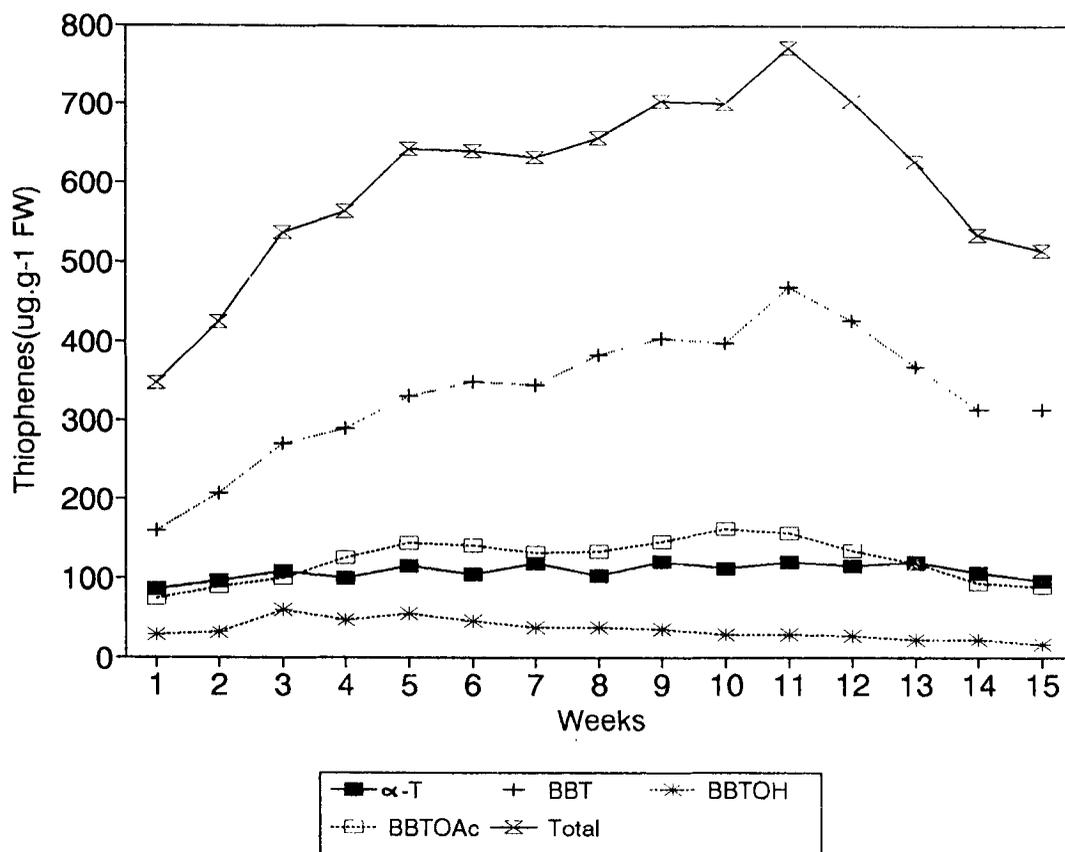


Fig. 3. Thiophene content in shoots of *T. argentina* along 15th weeks of culture ($\mu\text{g/g FW}$).

length detector connected to a Hewlett Packard 3396 integrator.

Both TLC and HPLC determinations were made at room temperature. All chemicals were of HPLC grade.

For thiophenes derivatives quantitation, stock solutions of each standard were prepared and calibration curves were made. The amounts of thiophenes compounds were measured by comparing their areas from the chromatograms with those of the standards. Volume injection was of 20 μl and each injection was repeated 3 times. BBTOAc and BBTOH standards were kindly supplied by Dr. J Lam (University of Aarhus, Denmark) BBT and $\alpha\text{-T}$ were purchased by Aldrich.

RESULTS AND DISCUSSION

The distribution of thiophenes was studied for 15 weeks along the ontogenetic cycle of *T. mendocina* and *T. argentina*. *T. mendocina* remained in the veg-

etative stage during the studied period but flowered in the 15th week. *T. argentina* reached the flowering stage in the 12th week.

The four studied metabolites are the main thiophenes present in *Tagetes* species. HPLC patterns revealed quantitative differences between roots and the vegetative shoots in glasshouse plants of both species in dependence of the developmental stage.

Considering the total thiophene concentration, the highest amount in the whole plants of *T. mendocina* was found during the 8th week and in *T. argentina* in the 11th one (Fig. 1 to 4). The total thiophene content in whole plants range from 0.5 $\text{mg}\cdot\text{g}^{-1}\text{FW}$ to 4 $\text{mg}\cdot\text{g}^{-1}\text{FW}$. Tosi *et al.* (1988) reported that *T. patula* reached the highest amount of thiophenes in whole plants during flowering and the yields range from 4 to 6 $\text{mg}\cdot\text{g}^{-1}\text{FW}$. It was found that the total thiophene content in *T. patula* reached the highest value about 80 days after germination while flowering occurred approximately at 60 days in hydroponically grown plants. (Downum and Towers, 1983).

Roots are the main site of thiophene accumulation in both species. They showed the highest total thiophene concentration at any stage of development in *T. mendocina* and *T. argentina* (Fig. 2 and 4). In both species, the total amount of the metabolites decrease towards the end of the studied cycle, mainly because of the decrease in the BBT level. The concentration of the other three thiophenes remain nearly constant in time. The decrease in the total concentration could be a consequence to the traslocation of the metabolites from the shoots and roots to the achenes.

BBT was the predominant thiophene either in shoots and roots of both species along the ontogenetic studied cycle (Fig. 1 to 4). Its level increases constantly and reach a peak content in the 11th week in *T. argentina*. Since that moment it decreases in concentration. The same is observed in *T. mendocina*, where the maximum value is found in the 8th week. Also BBT is the main thiophene in roots and shoots of *T. patula* during the ontogenetic cycle.

In *T. argentina* the highest thiophene formation occurred in the week previous to flowering. In *T. patula* the the highest value was found in roots (10-13 mg.g⁻¹ FW) in the full flowering plants, 50 days after germination (Tosi *et al.*, 1988). In *T. argentina*, BBTOAc is synthetized in roots in higher amounts until the 8th week, and α -T is prevalent from this week until the end of the cycle (Fig.4). In shoots, α -T is predominant up to the 3rd week, then its level is lower than BBTOAc until the 12th week and then increases again over BBTOAc until the 15th week (Fig 3). Except for the first 2 weeks, in *T. mendocina*, α -T is present in higher amounts than BBTOAc in roots and shoots over all the experimental period (Fig. 1 and 3). The concentration of BBTOAc, BBTOH and α -T remained nearly constant along the 15 weeks. Downum and Towers (1983) pointed out that the content of α -T changed very little over the experimental period, while BBT and BBTOAc are the main thiophenes in *T. patula*, increasing their concentration the first 80 days after germination.

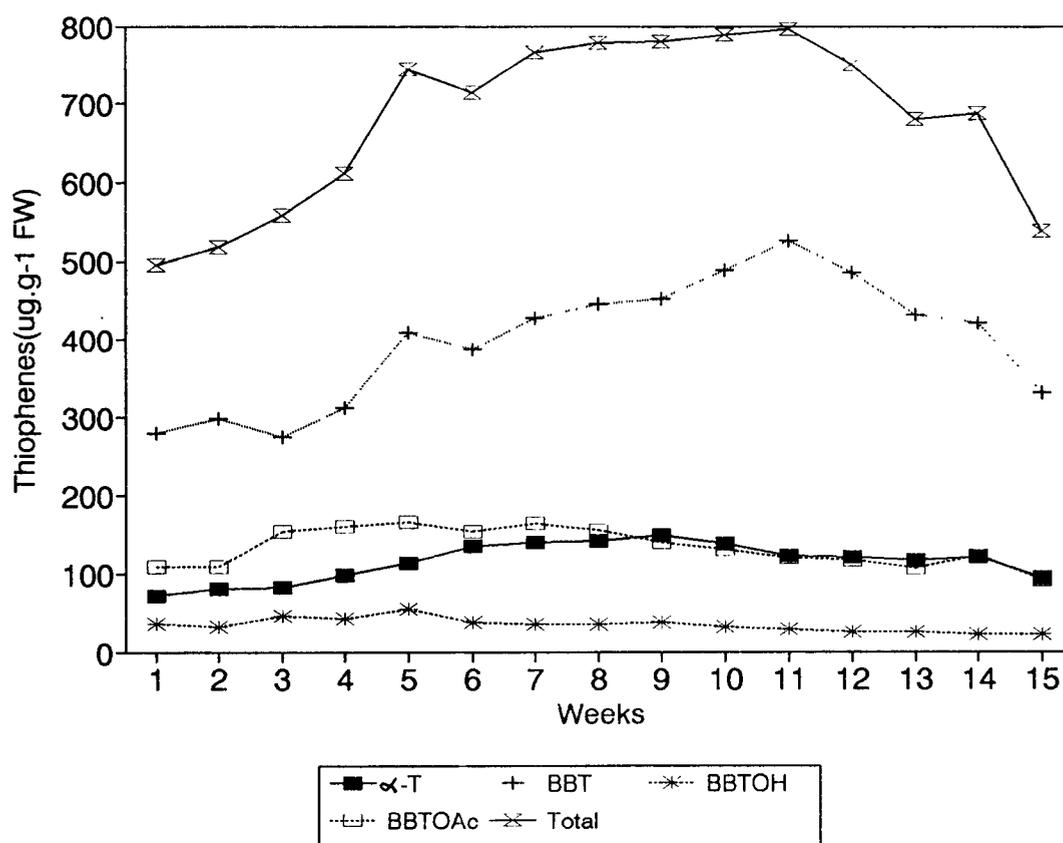


Fig. 4. Thiophene content in roots of *T. argentina* along 15th weeks of culture ($\mu\text{g/g}$ FW)

BBTOH is produced in low quantities either in shoots or roots of *T. argentina* and *T. mendocina* but in higher amounts in the first one. BBTOH is also found as a minor component throughout the plant in *T. patula* (Downum and Towers, 1983). In all plant organs, α -T and BBTOH do not increase appreciably in time in *T. patula* (Tosi *et al.*, 1988). The four individual thiophenes behave quite differently towards the end of the experiment. While BBT decrease in the whole plant in both species, the other three metabolites showed a little variation during all the studied cycle. Apparently, during ontogenesis there is a fluctuation in thiophene accumulation in different organs. In roots and shoots of *T. argentina* seems to be a stage dependent accumulation specially for BBT. In roots of *T. patula*, BBTOAc and BBT showed this stage dependent accumulation (Tosi *et al.*, 1988), the same phenomenon occurred with some alkaloids and other biologically active compounds (Kery, A., 1975; Johnson, A. *et al.*, 1985). The variation of the other studied thiophenes seems not to be related with the ontogenetic stage for both species. The total thiophene amounts found in these species are similar than those found in *T. patula* (Tosi *et al.*, 1988).

The significance of the distribution and accumulation of these compounds through the plant is not yet clear. Considering their defensive role in the plant, their accumulation may suggest a protection against herbivore attacks. Considering the light-induced nematocidal (Gommers, F., 1972; Arnason *et al.*, 1981a), insecticidal (Arnason *et al.*, 1981b) and antiviral (Hudson *et al.*, 1986b) activities demonstrated for α -T, their variation during the ontogenetic cycle may be related with a defensive role. However, their significance and fluctuation within different plant organs are far from being understood yet.

REFERENCES

- Arnason, T., G.F. Chan, C.K. Wat, K. Downum and G.H.N. Towers, 1981a. Oxygen requirement for near UV mediated cytotoxicity of α -terthienyl to *Escherichia coli* and *Saccharomyces cerevisiae*. Photochem. Photobiol. 33 : 821-825.
- Arnason, T., T. Swain, C.K. Wat, E.A. Graham, S. Partington, G.H.N. Towers and J.Lam, 1981b. Mosquito larvicidal activity of polyacetylenes from species in the *Asteraceae*. Biochem System Ecol. 9 : 60-63.
- Bakker, J., F.J. Gommers, I. Nieuwenhuis and H. Wyngberg, 1979. Photoactivation of the nematocidal compound α -terthienyl from roots of marigolds (*Tagetes* species). A possible singlet oxygen role. J. Biol. Chem. 254 : 1841-1844.
- Bohlmann, F. and C. Zdero, 1985. Naturally occurring thiophenes. In *Thiophenes and its derivatives*. Part I. Vol.44, p. 261, ed (S. Gronowitz), Wiley, New York.
- Bohlmann, F., T. Burkhardt and C. Zdero, 1973. *Naturally Occurring Acetylenes*. Academic Press, Inc., London. pp. 165.
- Chan, G.F., G.H.N. Towers and J.C. Mitchell, 1975. UV mediated antibiotic activity of thiophenes compounds of *Tagetes*. Phytochemistry 14 : 2295-2297.
- Downum, K.R. and G.H.N. Towers, 1983. Analysis of thiophenes in the *Tagetae* by HPLC. J. Nat. Products 46, 983
- Gommers, F.J. 1972. Increase of the nematocidal activity of α -terthienyl and related compounds by light. Nematologica 18 : 458-461.
- Hoagland, D.R., and D.I. Arnon, 1938. The water culture method for growing plants without soil. Calif. Agric. Coll. Expt. Stn. Circ. 347.
- Hudson, J.B., E.A. Graham, G.C. Chan, A.J. Finlayson and G.H.N. Towers, 1986a. Comparison of the antiviral effects of naturally occurring thiophenes and polyacetylenes. Planta Med 6 : 453-457.
- Hudson, J.B., E.A. Graham, N. Micki, L. Hudson and G.H.N. Towers, 1986b. Antiviral activity of the photoactive thiophene α -terthienyl. Photochem Photobiol 44 : 477-479.
- Johnson, A.E., R.J. Molyneux and G.B. Merrill, 1985. Chemistry in pyrrolizidine alkaloid content of *Senecio*, *Amsinckia* and *Crotalaria* species. J. Agric. Food Chemistry 33 : 50-55.
- Kery, A., 1975. Free and N-oxide alkaloids in various organs during the vegetation period. Acta Agron. Acad Sci. Hungaricae 24 : 3-6.
- Ketel, D.H., 1987. Distribution and accumulation of thiophenes in plant and calli of different *Tagetes* species. J. Exp. Bot. 38 : 67-61.
- Ketel, D.H. and H. Breteler, 1988. Morphogenesis and thiophene production in cell cultures of *Tagetes* species. In: *Chemistry and Biology of Naturally Occurring Acetylenes and Related Compounds*. Elsevier, Amsterdam. J.Lam, H. Breteler, T. Arnason and L. Hansen (Eds.), pp. 267-278.
- Norton, R.A., A.J. Finlayson and G.H.N. Towers, 1985. Thiophene production by crown galls and callus tissues of *T. patula*. Phytochemistry 24 : 719-724
- Sutfeld, R., 1982. Distribution of thiophenes derivatives in different organs of *T. patula* seedlings grown under various conditions. Planta 156 : 536-542
- Tosi, B., A. Donini, A. Bonora and A. Bruni, 1988. Accumulation and distribution of thiophenes in *T. patula*. Pharmacological Research Communications, Vol. 20, Supplement V : 117-122