Furthermore, this tissue culture loses its potential for flavonoid synthesis when grown as a cell-suspension in the same nutrient medium regardless of the carbon source. Considering its low auxin requirement, this tissue should lend itself favorably to nutritional and hormonal studies of the regulation of flavonoid synthesis in vitro[9].

Résumé. Les composés flavonoïdes d'une culture de tissu de vigne vierge cultivée en milieu Héller, contenant 5% de glucose et 0.1 mg/l d'acide naphtyl-acétique, ont été isolés et identifiés comme monoglucoside-3 et diglucoside-3 de quercétine et comme cyanine.

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1,2 Benzisothiazol-3-ylacetic Acid as a Growth Promoting Substance for *Helianthus tuberosus* (Jerusalem artichoke) in vitro

Experiments in vivo on a group of plants representative of the most common weeds, and on several plants of agricultural interest, have shown that 1,2 benziso-3-ylacetic acid (BIA), in addition to a strong phytotoxic action, has a remarkable selectivity for Graminaceae[1,2]. Besides this, it was observed that the herbicide action was accompanied with morphological modifications that are very similar to those induced by auxin. Recently it was demonstrated that BIA is characterized by many of the activities possessed by indol-3-acetic acid[3,4]. In fact it was observed that, as occurs for the natural auxins, BIA causes in the third internode of etiolated *Pisum satirum* a strong absorption of water, a considerable cell enlargement (pea test), a greater curvature of split internodes (split test), and moreover a notable production of ethylene.

From this point of view, and for the purpose of identifying more distinctly the biological activity of this new phytoactive molecule, we have subjected BIA to a series of tests for the induction of cellular multiplication in explants of *Helianthus tuberosus* cultivated in vitro.

Materials and methods. Explants of dormant tubers of *Helianthus tuberosus* (Jerusalem artichoke) var. OB1 were utilized. Cylindrical explants (3 mm diam., 4 mm height) of the homogeneous medullary parenchyma were placed in vitro in a nutritive medium[5] with glucose 5% and purified agar 1% (Fluka). 1,2 benzisothiazol-3-ylacetic acid (BIA) was used at molar concentrations between 10^{-4} and 10^{-7} with a control in basal medium alone and basal medium plus indol-3-acetic acid (IAA) at 2×10^{-6} molar concentration. BIA was obtained by synthesis as described[6], melting point: 153–4°C.

Fifteen replications were utilized for every concentration. The cultures were randomized in a culture room at 24°C in alternating light (3200 lux). The experiments were repeated twice at different times with similar results. Chlorophyll extraction and determination were made according to Smith and Benitez[7]; the contents were referred to fresh weight, corrected according to the surface.

Results and discussion. Observations made during the growth of the explants revealed that, already after 4 days from explantation, the tissues treated with 10^{-5} and 10^{-6} *M* BIA had visibly grown as much as when IAA was used. No growth was seen to occur at the other concentrations or in basal medium. After 6 days, the explants treated with 10^{-5} and 10^{-6} *M* BIA or 2×10^{-6} *M* IAA assumed a barrel-shaped form, whereas the tissues treated with 10^{-4} *M* and 10^{-7} *M* BIA began to grow also and showed a greater growth at the top or bottom of the explant, with the above concentrations respectively. The experiments were stopped after 12 days; fresh and dry weights and chlorophyll content were determined.

BIA has a very strong effect on cellular proliferation (Table), similar to that of IAA. The optimal concentrations 10^{-5} and 10^{-6} *M* cause an increase in fresh weight of 270 and 290% respectively and in dry weight of 199 and

[1] T. Vitali and L. Amoretti, Boll. Soc. Biol. sper. *47*, 790 (1971).
[2] T. Vitali, F. Mossini, R. M. Mingiardi, E. Gaetani and V. Plazzi, Acta nat. Ateneo Parmense 7, 71 (1971).
[3] C. Branca and E. Gaetani, Rc. Accad. naz. Lincei, Cl. Sci. Fis. Mat. Nat., in press (1973).
[4] T. Vitali, C. Branca and V. Plazzi, Boll. Soc. Biol. sper., in press (1973).
[5] F. Bertossi, N. G. Bot. ital. *66*, 497 (1959).
[6] Brevetto Italiano N. 852.483, 15 Novembre 1969; Ger. Offen. 1950.370, Chem. Abstr. *73*, 130991 (1970).
[7] J. H. C. Smith and A. Benitez, in *Modern Methods of Plant Analysis* (Ed. K. Peach and M. V. Tracey; Springer-Verlag, Heidelberg 1955), vol. 4, p. 142.

Effect of 1,2 benzisothiazol-3-ylacetic acid (BIA) on the growth of dormant tubers explants of *Helianthus tuberosus* in vitro

	Concentration (*M*)	Fresh wt. (mg)	Fresh wt. of control (%)	Dry wt. (mg)	Dry wt. of control (%)	Dry wt. (%)	Chlorophyll (µg/g fresh wt.)
	0	49.1 ± 4.2[a]	100	11.2 ± 2.4[a]	100	23.0	7.5
IAA	2×10^{-6}	141.6 ± 14.3	288	18.8 ± 1.8	167	13.3	5.8
BIA	10^{-4}	88.7 ± 4.8[a]	180	15.2 ± 1.6[a]	135	16.9	3.6
BIA	10^{-5}	135.0 ± 9.2	270	21.9 ± 3.0[a]	199	16.1	4.6
BIA	10^{-6}	146.5 ± 8.4	290	18.3 ± 2.7	166	13.1	4.7
BIA	10^{-7}	87.2 ± 17.2[a]	177	15.9 ± 2.9[a]	141	20.1	3.7

Average values ± SE were made on about 15 explants, 12 days old. The difference between each average and the control (basal medium alone) is significant at 1%; BIA 10^{-5} *M* and 10^{-6} *M* averages are significantly different at 1% (Student's *t*-test). [a] The difference of each average with the average of the explants treated with IAA is significant at 1% (Student's *t*-test). Chlorophyll content is corrected according to the surface.

166% respectively, in comparison with the control on basal medium. The data on the per cent of dry weight reveals that 10^{-6} *M* BIA is more similar to 2×10^{-6} *M* IAA, while 10^{-5} *M* BIA has lesser effect on the hydration. At optimal concentrations, BIA seems to inhibit the chlorophyll synthesis in respect to the IAA.

On the basis of the results obtained, it can be concluded that BIA acts not only on the cell enlargement as previously demonstrated[3], but also on cellular proliferation, which confirms the close analogy existing with the IAA, at very similar concentrations.

These results represent a further contribution to the characterization of the biological activity of the benzisotiazole compounds.

Riassunto. L'acido 1,2 benzisotiazol-3-ilacetico (BIA) ha mostrato una forte azione sulla proliferazione cellulare di espianti di tuberi dormienti di *Helianthus tuberosus*, sostanzialmente simile a quella indotta dall'acido indol-3-acetico.

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The Probable Basis of Adaptation in Mosquito Larvae

The role of predation as a major factor in natural selection has gained considerable importance, especially since the report of apostatic selection by CLARKE[1]. A number of papers have appeared on the influence of colour of the prey in predation. Individual fishes are known to exhibit strong preference for particular colours of prey (CLARKE, personal communications). It is the purpose of this note to point out such selective feeding mechanisms exhibited by 2 major predators on mosquito larvae, *Gambusia affinis* and *Culex* (*Lutzia*) *raptor*, and to compare the adaptive responses of 2 mosquito species with the available data on their genetic variabilities.

Given the choice in experimental plots, *Gambusia* feed preferentially on the larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex fatigans* in this order (Table I). However, when certain marker strains of the prey are used, feeding preference is to yellow or golden colored larvae irrespective of the species involved. From Table II, it may be seen that the 'golden' of *Culex fatigans* is preferred over the 'black' mutant of *Aedes aegypti* by *Gambusia*. Such 'nonspecies specific preference' to colour has been reported in *Culex* (*Lutzia*) *raptor*[2] also. These observations suggest that natural selection, through predation, acts unfavourably on the pale mutant forms since they are predated upon preferentially. On the other hand, the darker colour which has a protective adaptation is of selective advantage in field populations of mosquito larvae where the incidence of predation is high.

Such incidences of visual selection by the predators could exert apostatic adaptation in the prey[1], and it has been recently concluded that, under specific conditions, a polymorphic population may have a larger number of individuals than a monomorphic one[3]. Colour polymorphism is very well known in mosquitoes. *Aedes aegypti*, for example, is known to exhibit a wide spectrum of colour variations from pale yellow forms at one end to very dark ones at the other. The theory of domesticity and paleness[4] accounts for certain probable factors tending the selection of pale colour in the laboratory; but data on the probable causes of favourable selection of dark colour in the field are not available. It is generally known that larvae freshly collected from the field are darker than the laboratory strains. The maintenance of such polymorphism by selection could be brought about by keeping certain gene combinations in particular ratios of selective advantage to others[5]. In other words, the response to such selection pressures would depend to a major extent on the genetic variability and hence adaptability of the species involved. In *Aedes aegypti*, a balanced polymorphic system for loci *y*, *s*, *ds*, and another mutant Gold which are linked fairly closely has been suggested[6]. The maintenance of different advantageous colours in varied selective pressures in different populations could be accounted for by such a polymorphic system. However, even such balanced polymorphic loci require different fitness values for various genotypes[7] and the accommodation of such systems in animals that have a higher genetic variability could be more directly proportional.

Table I. Feeding behaviour of *Gambusia* in a 'choice' experiment involving the 3 species of mosquito larvae

	Initial No.	12 h	24 h	36 h	48 h	Total consumed
A. aegypti	200	168	141	107	85	115
An. stephensi	200	187	176	167	158	42
C. fatigans	200	194	189	183	176	24
G. affinis	6	6	6	6	6	0

Table II. Feeding behaviour of *Gambusia* in a 'choice' experiment involving mutants of *Aedes* and *Culex*

	Initial No.	12 h	24 h	36 h	48 h	Total consumed
A. aegypti (black)	200	189	181	160	161	39
C. fatigans (golden)	200	167	134	98	69	131
G. affinis	6	6	6	6	6	0

1 B. CLARKE, in *Taxonomy and Geography* (Ed. D. NICHOLS; Syst. Ass., Oxford 1962).
2 P. T. RAJASEKHARAN and B. N. CHOWDAIAH, Experientia *28*, 981 (1972).
3 B. CLARKE, Am. Nature *106*, 1 (1972).
4 G. B. CRAIG, R. C. VANDEHEY and W. A. HICKEY, Bull. Wld. Hlth. Org. *24*, 527 (1961).
5 A. J. CAIN and P. M. SHEPPARD, Am. Nature *88*, 321 (1954).
6 G. A. H. MCCLELLAND, in *Genetics of insect vectors of disease* (Eds. J. W. WRIGHT and R. PAL; Elsevier Pub. Co., Amsterdam 1967).
7 K. KOJIMA, Th. Pop. Biol. *2*, 159 (1971).