Effect of terpenoids on the root formation of *Phaseoulus aureus*

		Concentration (ppm) 5 ppm	10 ppm	15 ppm	20 ppm	25 ppm
		Number of roots				
Isopatchoulenone	(I)	6.0±1.2	26.8±1.4	33.7±1.3	43.0±1.8	54.6±1.4
Cyperene	(II)	5.6±1.4	6.4±1.2	7.2±1.2	6.8±1.4	7.3±1.1
Piperitenone	(III)	21.0±1.7	34.6±1.1	17.0±1.4	23.5±1.5	28.5±1.8
Piperitone	(IV)	31.2±1.3	28.7±1.4	27.5±1.5	20.5±1.3	18.2±1.3
Carvone	(V)	5.6±1.7	6.6±1.3	7.8±1.3	5.6±1.5	6.4±1.2
Pulegone	(VI)	15.6±1.2	17.0±1.3	23.8±1.1	17.0±1.3	14.6±1.6
α-Ionone	(VII)	11.0±1.3	9.7±1.6	10.0±1.7	11.2±1.3	13.5±1.2
β-Ionone	(VIII)	6.6±1.2	25.2±1.3	24.0±1.4	21.6±1.3	15.5±1.5

Mung bean rooting tests were performed in the laboratory using the basic methodology of Hess[6]. IAA was dissolved in minimum quantity of ethyl alcohol and the dilutions were made with distilled water to give a standard ppm solution for control reference. Control experiments: H_2O: 7.6±1.4; IAA (10 ppm): 16.7±1.8.

structure-activity data a large scale screening of essential oils and their component terpenoids was undertaken in this laboratory for their evaluation as plant growth regulators.

The essential oil from the tubers of *Cyperus scariousus* significantly induced the formation of roots on the stem cuttings of *Phaseoulus aureus.* A major identified terpenoid constituent of this oil is the crystalline α,β-unsaturated ketone isopatchoulenone[5] (**I**). Our earlier findings[4], that structurally related cross-conjugated terpenoid ketones stimulate root formation, suggested that the biological activity of the essential oil may be due to **I.**

These data prompted us to evaluate α,β-unsaturated terpenoid ketones for regulating growth in plants. The work reported in this paper shows that, with the glaring exception of carvone (**V**), α,β-unsaturated ketones cause adventitious root formation in the hypocotyl cuttings of *P. aureus.* At suitable concentrations (table), several conjugated ketones were found to be more potent to cause root formation over IAA. Significantly isopatchoulenone (**I**) in concentrations above 10 ppm was more prominent in displaying this effect.

This is to our knowledge the first demonstration of root-forming ability of α,β-unsaturated terpenoid ketones. To confirm that the plant growth activity in **I** is due to α,β-unsaturated ketone moiety was revealed when the root promoting activity fell off in cyperene (**II**), which is the parent hydrocarbon of **I**, and is present in the same essential oil as another major component.

A comparison (table) of stimulation of rooting by **I** with IAA and other conjugated ketones suggests that the extent and position of substitution of enone chromophore, together with the endo- or exo-position of the double bond in the ring, may be the deciding factor for this action. Interestingly like[4] (**IX** and **X**) α-ionone (**VII**) with a disubstituted double bond is only slightly active over control experiments. With extended conjugation its isomer β-ionone (**VIII**) regains the root-inducing potential which is more than IAA at 10 ppm.

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Absorption and translocation of tetrachlorodibenzo-p-dioxine by plants from polluted soil[1]

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Summary. Measurements were made of contamination of plants grown in soil polluted with tetrachlorodibenzo-p-dioxine. Findings show that the pollutant is absorbed and translocated by the plants studied and suggest that the pollutant may be eliminated in the course of time.

After the explosion of the trichlorophenol reactor at Seveso in northern Italy[2] which caused an escape of tetrachlorodibenzo-p-dioxine (hereafter referred to as TCDD dioxine), which is a toxic (teratogenic and acnegenic[3,4]) substance, a knowledge of intake, accumulation and metabolization of TCDD by higher plant life came to be of vital importance. The possible ability of plants to take in and accumulate dioxine might represent a danger in spreading pollution; furthermore, their ability, if any, to take in, accumulate and eliminate dioxine might contribute to resolving the problems of decontamination; it might, for example, be the only solution to the problem of reclaiming extremely extensive areas of land with a low level of pollution.

Isensee and Jones[5] have experimented with oat and soya plants, showing that dioxine does not appear to interfere with growth of the plant to maturity, and that it is found in limited quantities in the tissues and does not appear to accumulate. Their studies also reveal that TCDD concentration in plants appears to reach a maximum level and then to decrease with time, particularly in the aerial part of the plant. Isensee and Jones suggest a removal by metabolization, volatilization from the tissues or backward transport to the roots.

The possibility that plant life may in some way degrade dioxine has been suggested by US Air Force research workers, who have shown that, when plants are treated with

^{14}C-labelled dioxine, the radioactivity is not completely recuperable in the form of dioxine, part of the ^{14}C radioactivity apparently being incorporated into the plant tissues. Crosby et al.[6] have shown that dioxine present on foliage undergoes rapid photochemical dechlorination, suggesting that, if dioxine is absorbed by the plant and translocated to the leafage, it may then be eliminated by the action of light. The high level of contamination in the Seveso area provided an opportunity to study the relationships between dioxine and vegetation, and in particular to make a study on material present in the area, of the level of pollution of plant organs and tissues formed during the spring following the explosion, so as to bring to light any phenomena of uptake, accumulation and elimination of dioxine.

Methods. Plant material was collected in the Seveso area at points having a pollution level from 1000 to 400 µg per m^2. Sampling was carried out in such a way as to minimize the risk of pollution. Aerial portions were taken only when there was the certainty that they had no contact with soil, dust or water containing dioxine. Underground portions were removed from the soil, washed repeatedly, and then, in the laboratory after a further wash, tissue separation was carried out. Soil sampling was carried out by cylindrical sections (10 cm in diameter, 14 cm in length) made in points corresponding to the angles of an equilateral triangle of variable size within the area from which plant material was collected. Extraction and dosage of dioxine were carried out both on soil samples and on plant material following the methods described by Baughman et al.[7] and further elaborated by Baughman with modifications by Cavallaro et al.[9]. Soil and plants were extracted with hexane:acetone (4:1); the extract was concentrated and dehydrated, successively purified on multilayer silica gel column (H_2SO_4 on celite-$NaHCO_3$-Na_2SO_4 silica gel) fluorisil and lastly on a Brockman alumina microcolumn. The dried eluate in iso-octane then underwent dosage by mass GC-fragmentometric analysis at m/e 320-322-324. This procedure permitted purification of all materials analyzed, so as to avoid any mass interference.

Results and discussion. The results obtained provide an evaluation of dioxine content of prepollution and recently-grown parts of trees, ornamental and garden plants in the polluted zone of Seveso.

Samples were taken from cherry, fig, pear, apricot, peach trees and from vines in areas with differing levels of pollution. These samples were of new leaves and fruits formed in the year after dioxine pollution. In some cases, samples were also made of the twigs responsible for the new vegetative activity and of cork from the trunk, these being materials which already existed when pollution took place. Results show in all cases that dioxine is present in new leafage and fruits, pollution being from 3 to 5 times higher in foliage than in fruits. Twigs appear to have a higher dioxine content than is to be found in the inactive cork tissue, which had also been exposed to dioxine pollution in the summer of the year before. For the cherry tree, the dioxine content expressed as µg per kg fresh weight was: leaves 1.01; fruits 0.395; twigs 13.14; cork 3.16. Similar results were obtained for the other plants already mentioned. Concentration of dioxine appears to be higher in the leaves, which have a higher rate of transpirational flow, than in the fruit; dioxine level is higher in the twigs, in which transport take place, than in the cork, which is an inactive tissue; dioxine is found at higher concentration in young twigs and the cork than in leaves and fruit. These findings show above all that dioxine is translocated in newly-formed organs and suggest that the lower concentration in fruits and leaves may be due to same form of elimination such as dispersion in the transpiratory flow or photodestruction due to UV-rays, in agreement with the findings of Crosby et al.[6]. In the spring following the pollution, specimens were collected of kitchen-garden plants such as the carrot, the onion, the potato and the narcissus. In the case of the horticultural plants, the new vegetative activity was sustained by underground organs formed in the preceding year by plants which had undergone the toxic rain. In the case of narcissus, growth was sustained by underground organs which had been formed and had entered into quiescence before pollution. The dioxine content of the underground organs of the former group of plants could partly be due to a translocation of dioxine from leaves after pollution; for narcissus it can certainly be referred exclusively to intake of dioxine from the soil when the growth cycle began again. Underground organs in any case always appear to contain dioxine with values at the maximum equal to those of the soil (as µg per kg). The outer parts in contact with the soil, in comparison with the inner parts, show differences that cannot be correlated with a soil contamination. A comparison between the results obtained with the potato tuber and the carrot taproot showed that the highest dioxine content was to be found in correspondence with the arrangement of the conductive tissues. In the potato, the outermost tissue, in which conductive tissues are prevalent, had a dioxine content 30% higher than that of the inner parenchyma; in the carrot the central internal cylinder showed a dioxine content 50% higher than that of external parenchyma; in bulbs the external and internal dioxine content was the same. In all plants examined, the new aerial portions appeared to contain less dioxine than the underground organs (table 1). These findings show the ability of the plants to take in dioxine and to translocate it to aerial parts, probably through the conductive vessels; the lower dioxine

Table 1. Dioxine content of carrot, potato, onion and narcissus plants

	Aerial parts	Underground parts		Soil
		Inner parts	Outer parts	
Carrot	2.150	4.462	9.203	5.310
Potato	2.115	3.560	1.961	8.330
Onion	0.835	1.798	1.763	2.680
Narcissus	1.658	2.221	2.593	4.370

Values are expressed as µg per kg fresh weight and as µg per kg fresh soil. Values are the mean of 2 determinations.

Table 2. Decrease of dioxine content of carrot, potato, onion and narcissus plants after transplantation out of polluted soil in unpolluted soil

	Dioxine content at the of transplantation out of polluted soil		Dioxine content after the time in unpolluted soil	
	Aerial parts	Underground parts	Aerial parts	Underground parts
Carrot*	-	8.90	NV	0.760
Potato*	-	1.86	NV	NV
Onion**	0.985	2.26	NV	0.062
Narcissus***	-	2.62	0.019	0.022

NV: Not valuable. Values are expressed as µg per kg fresh weight. Values are the mean of 2 determinations. * Transplanted without aerial parts and harvested 4 months after. ** Transplanted with aerial parts and harvested 4 months after. *** Transplanted without aerial parts and harvested 10 months after.

content of the aerial parts suggests, as for the trees, that there is some possibility of its being eliminated. Furthermore carrot taproots, potato tubers, narcissus bulbs and whole onion plants were first washed several times and then moved to unpulled soil. 4 months after this transplantation (10 months for narcissus bulbs) the whole plants were harvested and analyzed for dioxine content in both aerial and underground organs. Data showed the disappearance of dioxine during this period from the underground organs and from aerial organs of old formation, and its absence from newly-formed aerial parts (table 2). If we exclude the possibility of the dioxine having been transported backward to the soil, we may formulate 3 hypotheses: translocation of dioxine to aerial parts followed by photochemical degradation due to sunlight, as suggested by Crosby's findings; translocation from underground to aerial organs and dispersion of the pollutant into the external environment by transpiration; metabolization by the plant. Experiments are now being carried out to test these hypotheses.

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Effet du ribonucléate de sodium sur la croissance et l'activité hémolytique de *Treponema hyodysenteriae*

Effect of sodium ribonucleate on the growth and the hemolytic activity of *Treponema hyodysenteriae*

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Summary. Liquid cultures of different strains of *Treponema hyodysenteriae*, when supplemented with sodium ribonucleate show an increase in the hemolytic activity titers while the number of colony forming units remain constant.

Un spirochète anaérobie, *Treponema hyodysenteriae*, est reconnu à l'heure actuelle comme l'agent étiologique primaire de la dysenterie porcine[2-4]. Ce tréponème diffère des autres tréponèmes cultivables in vitro non seulement par le fait qu'il est pathogène, mais aussi parce qu'il est hémolytique. Récemment, certains auteurs[5,6] ont démontré que les porcs sont porteurs d'un tréponème qui, par sa morphologie et sa mobilité, est confondu avec *T. hyodysenteriae*. Les seuls critères permettant de différencier ces 2 types de tréponèmes sont le pouvoir entéropathogène et le patron hémolytique observé sur des géloses au sang. Selon ce dernier critère, on peut différencier les souches de tréponèmes en fortement et faiblement β-hémolytiques. Il a été démontré qu'il existe une corrélation positive entre le pouvoir hémolytique et l'entéropathogénicité des souches[6]; seules celles fortement β-hémolytiques sont entéropathogènes. Des études préliminaires nous ont permis de mettre en évidence que cette activité hémolytique, insensible à l'oxygène, était produite en milieu liquide. En nous inspirant de travaux effectués avec la streptolysine S[7] et l'aérolysine[8] nous avons testé l'effet du ribonucléate de sodium sur la production de cette activité hémolytique, car l'obtention d'un titre hémolytique élevé est une condition préalable à tout essai ultérieur de purification. Les résultats ayant permis de démontrer que le ribonucléate de sodium augmentait la production de l'activité hémolytique, nous avons donc recherché la concentration optimale de ribonucléate pour la production de cette activité ainsi que son influence sur la croissance des tréponèmes.

Matériel et méthodes. Les souches de tréponèmes utilisées sont les suivantes: *T. hyodysenteriae* ATCC 27164, PM_9 et PA_2. Les souches PM_9 et PA_2 ont été isolées dans nos laboratoires à partir du colon d'un porc mort de dysenterie (PM_9) et de matières fécales provenant d'un porc sain (PA_2). L'isolement des souches a été effectué sur milieu Trypticase Soy Agar (TSA, Baltimore Biological Lab., USA) additionné de 5% de sang bovin citraté et de 400 µg par ml de chloro-pentahydrate de spectinomycine (Upjohn Co.) tel que décrit par Songer et al.[9]. Sur ce milieu, les souches ATCC 27164 et PM_9 apparaissent fortement β-hémolytiques alors que la souche PA_2 est faiblement β-hémolytique. Les souches sont maintenues par repiquages sur le même milieu TSA sans spectinomycine et incubation à 37 °C dans des jarres anaérobies Gas pak contenant une atmosphère de 80% d'H_2 et 20% de CO_2, tel que décrit par Kinyon et Harris[10], ou en bouillon SB-BHI composé de parties égales des milieux Spirolate (SB, Baltimore Biological Lab.) et Brain Heart Infusion (BHI, Baltimore Biological Lab.) tel que décrit par Saheb et Richer-Massicotte[11]. Les milieux sont pré-réduits et stérilisés dans une atmosphère d'azote désoxygéné tel que décrit par Kinyon et Harris[10]. Avant l'emploi on rajoute aux milieux 10% de sérum de veau fétal (Grand Island Biological Co.) inactivé 30 min à 56 °C. Les précultures sont préparées en ensemençant 10 ml de milieu liquide avec une colonie isolée, prélevée sur une gélose au sang, et incubation à 37 °C. A partir de précultures de 4 jours on ensemence, en utilisant des inocula d'un ml, des volumes de 20 ml des milieux décrits plus haut contenant, le cas échéant, du ribonucléate de sodium (*Torula* yeast, Sigma) stérilisé par filtration. On suit la croissance à intervalles réguliers par un comptage d'unités viables qui est effectué par étalement de volumes appropriés des dilutions convenables en PBS (tampon phosphate 50 mM, pH 7,0; NaCl 140 mM) sur des géloses