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## Effects of phthalimide on growth and alkaloid formation of *Datura metel* L.

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**Summary.** When administered to *Datura metel* L. by foliar or root application, the new plant growth regulator phthalimide exercises a favorable effect on the vegetative growth and also stimulates the formation of tropane alkaloids.

**Key words.** *Datura metel*; phthalimide; vegetative growth; alkaloid content.

Recently, phthalimide and its related compounds have been reported to be a novel group of chemicals which influence a variety of growth and development processes in many plant species<sup>1</sup>. *Solanaceae* in particular (*Solanum esculentum* Mill. and *S. tuberosum* L.) are very responsive. However, no information is recorded on the influence of phthalimides upon medicinal plants. In this context, in the course of research to study the morphophysiological effects induced by new plant growth regulators in *Solanaceae* synthesizing tropane alkaloids, we tested phthalimide (PTL) on *Datura metel* L. The investigation presented here was made to discover how far PTL could influence the growth and the synthesis of secondary products in this important solanaceous plant.

**Materials and methods.** Uniform size seedlings of *Datura metel* L. (25 days old) were employed in this study. The method of seed germination and the soil composition are described in a previous publication<sup>2</sup>. PTL, employed in water solution as potassium salt, was administered by foliar spraying and/or by root immersion. Foliar treatment was made by spraying PTL solutions (700 and 1400 µg/ml) containing 0.6% Tween 20 by a small pressure pump at a rate of 10 ml per plant. The plants received two sprays at 10 and 20 days after transplanting. Root immersion treatment was carried out by dipping the organs for 5 sec in a solution containing 4000 µg/ml of PTL immediately before transplanting. 50 pots were used with 10 samples for each concentration of PTL as well as a control at the onset of the experiment, which was carried out in the greenhouse of the Pharmaco-Biological Department of the University of Messina (Italy).

At 30 days after transplanting, the plants were harvested and the following parameters were recorded: plant height, stem diameter, number of leaves, dry weight, alkaloid content. Height measurements were taken from the soil line to the highest apex. Stem diameter was determined by measuring the circumference of the main stem at the soil line, at the top below the first main 'Y' branch, and midway between these two points. The average of these three readings was taken as the stem di-

ameter. For fresh and dry weight measurements, the plants were divided into leaf-tops, stem and root portions and the fresh weight of each portion was determined immediately. Dry weight as a percentage of fresh weight was determined after drying for 48 h in a forced-air drier at 48.5°C<sup>3</sup>. For alkaloid estimation, samples from dry plant organs were powdered (to 60 mesh). Alkaloids were extracted with a mixture of chloroform and peroxide-free ether (1:3). The extract was evaporated to dryness on a water-bath and the residue dissolved in a few milliliters of chloroform and 0.02 N sulphuric acid. The excess of acid was titrated with 0.02 N sodium hydroxide using methyl red mixed solution as indicator. The total alkaloid content, expressed as hyoscyamine, was determined from the expression given in the European Pharmacopoeia<sup>4</sup> and the results were calculated on a dry weight basis.

**Results and discussion.** As seen in the table, PTL-treatment exercised a marked stimulatory action upon the vegetative growth of *D. metel*. The effect was observed when PTL was administered both by foliar and root applications. At harvest, all treated plants were taller than the controls (fig. 1) and showed a marked internode elongation. The greatest increase in height occurred in plants sprayed with 1400 µg/ml of PTL. This concentration also induced the major increase in stem diameter. In the presence of PTL the number of leaves was greater, and the stimulatory effect was weakly higher when the chemical was administered throughout the roots. However, in the PTL-treated plants no difference was noted with regards to the size and morphology of the leaves, or the formation of lateral buds and shoots. Furthermore, under our experimental conditions PTL was completely non-toxic for *Datura metel* plants. This conclusion is supported by the observations of increase in dry weight. In fact, from data in the table it can be observed that PTL exercised a favorable effect also on the increase in dry weight in all the tested organs of *Datura metel* plants. Moreover, also in this case the major increase occurred when a solution containing 1400 µg/ml of PTL was sprayed on the leaves.

Effect of phthalimide on vegetative growth of *Datura metel* L.<sup>1</sup>

Treatments (µg/ml)	Stem length		Stem diameter		Leaves		Dry material <sup>2</sup>		Stems		Leaf-tops	
	cm	% of control	cm	% of control	No.	% of control	Roots	% of control	g	% of control	g	% of control
Foliar spraying												
Control	29.35 ± 0.9	—	0.65 ± 0.03	—	14.34 ± 0.5	—	15.53 ± 0.2	—	13.19 ± 0.2	—	14.22 ± 0.4	—
700	40.12 ± 1.5	136.69 ± 9.2	0.84 ± 0.02	129.23 ± 9.1	17.63 ± 4.0	122.94 ± 7.1	17.32 ± 0.2	111.53 ± 2.7	15.36 ± 0.3	116.45 ± 4.0	18.05 ± 0.6	117.58 ± 6.8
1400	48.66 ± 1.9	165.79 ± 11.5	0.92 ± 0.05	141.54 ± 14.2	18.47 ± 0.7	128.80 ± 9.4	20.07 ± 0.1	129.33 ± 2.3	18.30 ± 0.3	138.74 ± 4.6	16.72 ± 0.5	126.93 ± 7.8
Root immersion												
Control	27.06 ± 0.5	—	0.53 ± 0.02	—	14.26 ± 0.5	—	15.08 ± 0.1	—	12.80 ± 0.2	—	13.88 ± 0.3	—
4000	36.28 ± 1.2	134.07 ± 6.9	0.85 ± 0.03	134.92 ± 9.1	18.58 ± 0.6	130.29 ± 9.2	19.21 ± 0.2	127.39 ± 2.2	16.65 ± 0.3	130.08 ± 4.4	15.75 ± 0.5	113.47 ± 6.1

<sup>1</sup> Values are mean ± SE of four determinations. <sup>2</sup> Calculated as % fresh weight.

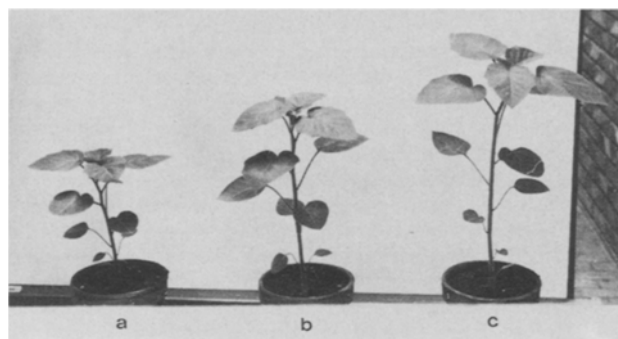


Figure 1. Effects of phthalimide on the vegetative growth of *D. metel* L. The plants were treated by foliar irrigation at 10 and 20 days after transplanting (for details see text). a, control; b, 700 µg/ml; c, 1400 µg/ml.

As shown in figure 2, PTL-treatment stimulated alkaloid formation in the aerial parts of *D. metel* plants. On the other hand, in the roots the total content of the active principles declined. In any case, however, the induced decrease was lower than the increase that resulted at stem and leaf-top levels. The effects were dose dependent and the major increase was obtained in the plants that were sprayed with 1400 µg/ml of PTL. From the above results it may be concluded that PTL treatment has a stimulatory effect on the vegetative growth of *D. metel* that is accompanied by an augmented synthesis of tropane alkaloids. It is well established that in the herbaceous *Datura* the active principles are synthesized mainly in the roots, from which they are translocated to the aerial parts<sup>5</sup>. In our case, the observed decrease of the alkaloids in the roots and the increase in the leaves and stems following PTL-treatment led to the conclusion that in *Datura metel* the growth regulator stimulates the formation of alkaloids in the roots and also stimulates the transportation of these metabolic products to the aerial parts of the plant, where they accumulate.

On the basis of our data it is not possible to explain how PTL induces these effects. The mechanism of action of PTL is completely unknown and its interaction with the metabolic processes of the plant is still to be studied. Furthermore, any comparison with other tested compounds is difficult, since the responses of the genus *Datura* to the various tested plant growth regulators are very variable depending upon the species, the nature and the concentration of the chemical applied, the method and frequency of application, the stage of plant development when the applications are made and the period of

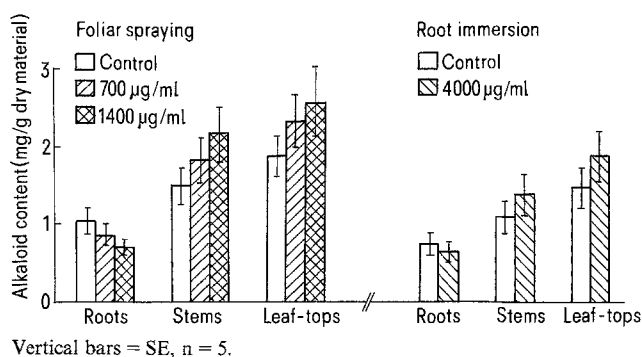


Figure 2. Effect of phthalimide on alkaloid content (total alkaloids calculated as hyoscyamine) of *D. metel* L. plant organs. The determinations were made on pooled samples of four plants per group.

time which has elapsed between the time of application and the harvest<sup>5,6</sup>. However, whatever the action mechanism of PTL in *D. metel* may be, the results here reported are to be considered of some interest, as a stimulatory effect on growth is rarely accompanied by a significant augmentation of alkaloid content in *Datura*<sup>7-9</sup>.

So far, the results here reported are encouraging and this study gave convincing evidence for the usefulness of the application of PTL to *Datura metel*.

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## Karyotypes of two species of Insectivora from Taiwan (Insectivora, Soricidae)

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**Summary.** The karyotypes of two Insectivora species from Taiwan are described here for the first time. *Soriculus caudatus fumidas* has  $2n = 40$  chromosomes,  $FN = 52$  and *Anourosorex squamipes yamashinai* has  $2n = 50$  chromosomes,  $FN = 96$ . For *A.s.yamashinai* the G- and C-banding pattern are presented.

**Key word:** Karyotype; G-banding; C-banding; Insectivora.

The genus *Soriculus* consists of about six species distributed over Bhutan, Kumaon, Sikkim, Nepal, China, North Burma, Tonkin and Formosa. The genus *Anourosorex* consists of a single species only ranging from Indo-China to Formosa<sup>2</sup>. No species of either genus has been karyotyped so far. The present paper reports the karyotypes of *Soriculus caudatus fumidas* and *Anourosorex squamipes yamashinai*.

**Materials and methods.** *Soriculus caudatus fumidas* and *Anourosorex squamipes yamashinai* collected from Mt. Ari, Taiwan were used for the present study. They were classified following the checklist of Ellerman and Morrison-Scott<sup>3</sup>. Cytological preparations were made from primary lung tissue cultures using the standard air drying method. The G- and C-band techniques of Seabright<sup>4</sup> and Sumner<sup>5</sup> were applied.