

Chlorea rigidula Stirt., which gets nomenclatural priority in the following new combination:

Usnea rigidula (Stirton) G. Awasthi, comb. nov. —

Chlorea rigidula Stirt., Scott. Natur. 7:75 (1883).

Type: India, Nilgherries (Nilgiri), G. Watt 13, pr. maj. p. (Lectotype here proposed: BM!)

TLC: usnic acid and salacinic acid.

— *Usnea ceylonica* Mot., Lich. Gen. Usnea Stud. Monogr. Pars Syst. :129 (1936–38). Type: Ceylon (Sri Lanka, no precise locality), Rietzner (Holotype: UPS – not seen). Authentic specimens seen: Ceylon (Sri Lanka), Thwaites 17 (BM!, UPS!).

TLC: usnic acid and stictic acid complex (stictic acid, constictic acid, norstictic acid in trace, and grey spot below stictic acid at R_f value 0.26 in solvent A).

— *Usnea venosa* Mot., Lich. Gen. Usnea Stud. Monogr. Pars Syst. :474 (1936–38). Type: Herb. Ind. Or. (no precise locality), Hook. f. & Thomson 1718 B (Holotype: W!; isotype W!).

TLC: usnic acid, salacinic acid and an unknown yellow spot with greenish rim at R_f value 0.46 with grey spot below it in solvent A in holotype specimen; isotype has usnic acid and salacinic acid only.

Several specimens collected in recent years from Nilgiri and Palni hills were found to correspond with the taxon *Usnea rigidula* (Stirt.) G. Awasthi in morphology and anatomy, but with three distinct chemical strains: usnic acid and salacinic acid strain; usnic acid, salacinic acid, unknown yellow spot strain, and usnic acid and stictic acid complex strain. *Usnea rigidula* is distinctive in its rigid isotomic dichotomous branching, irregularly cracked pseudocyphellate cortex, dense medulla and thick axis.

A specimen collected from Himalayas by Hooker and Thomson, no. 1718 (GLAM, BM), was considered close to *Usnea longissima* Ach. by Stirton⁵ and remarked "the axis is a brownish or violaceous black colour, and it gives a negative reaction with I. This lichen is also much more robust than usual, and has a crisped appearance. To this I propose giving the name *Usnea mekista*. It certainly deserves the rank of a subspecies". This clearly indicates that Stirton considered the taxon as only a subspecies of *U. longissima*. But Motyka³ described it as a species without the formal combination in the specific rank. The formal new combination is therefore given below:

Usnea mekista (Stirton) G. Awasthi, comb. et stat. nov. — *Usnea longissima* subsp. *mekista* Stirt., Scott.

Natur. 6:105 (1881). Type: Himalayas (no precise locality), Holotype: Hooker and Thomson 1718 (GLAM!); isotype (BM!).

TLC: usnic acid and fumarprotocetraric acid.

The taxon is distinct from *U. longissima* Ach. in the 5–10 mm long crisped lateral branchlets, filamentose branches annularly cracked, more or less pulverulent and with isidiate and sorediate verrucae on the cortex, the central axis violet brown and negative in reaction to iodine solution.

I am thankful to the Directors and the Keepers of the several herbaria mentioned in the text for the loan of the specimens referred to above. I am thankful to Dr F. R. Woodward for the information mentioned above. For guidance and helpful discussions, I am grateful to Dr B. B. Sharma and Dr D. D. Awasthi. This work has been carried out under a fellowship grant to me from the Botanical Survey of India.

13 August 1984; Revised 5 November 1984

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DICHLOBENIL ARRESTS ROOT HAIR GROWTH IN RADISH

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DICHLOBENIL (2,6-dichlorobenzonitrile), a broad-spectrum herbicide¹, is a potent inhibitor of cellulose synthesis^{2,3}, and pollen tube growth⁴. Like pollen tube, root hair is characterized by tip growth. This paper reports the effects of dichlobenil on root hair growth in radish.

A stock solution of dichlobenil (98%, Tokyo Kasei Co. Japan) was prepared in boiling water and cooled to room temperature. A 1.4×10^{-4} M solution was nearest to the highest concentration of aqueous sol-

ution that could be obtained. The pH of the stock solution was adjusted to that of distilled water (6.35–6.55). Four concentrations of dichlobenil were tested: 10^{-7} , 5×10^{-6} , 5×10^{-5} and 1.4×10^{-4} M.

Seeds of radish (*Raphanus sativus* cv Pusa Chetki obtained from Indian Agricultural Research Institute, New Delhi) were soaked in tap water for 24 hr; by this period the seeds became fully imbibed but did not issue the radicles. Such fully imbibed seeds were sown on filter paper discs placed in petri dishes (diameter 15 cm) supplied with 10–15 ml aqueous solution of dichlobenil. Control batches of seeds were given distilled water. In each petri dish 10 or 20 seeds were sown. All the seed cultures were incubated at $28 \pm 2^\circ\text{C}$ in darkness.

Two days from incubation the response of seeds in a given treatment was nearly uniform with reference to germination and root hair production. Therefore, root hair growth was estimated in 2-day old seedlings by the stain-destain method devised by Beasley and Ting⁵ and slightly modified by Beasley *et al*⁶ for quantifying cotton fibre growth.

From each petri dish culture five seedlings were chosen at random and stained with 0.02% toluidine blue O (E. Merck) in dibasic sodium phosphate-citric acid buffer (pH 4.5) for 20 sec. The stained seedlings were washed thrice with distilled water. Radish seedlings are relatively hardy and are amenable to manual operations. To isolate the root hairs the stained seedlings were held with forceps on a microslide and their radicles were scraped with a surgical blade. However, the tiny root hairs near the tip of the radicle could not be severed. The isolated root hairs were destained in 10 ml formalin acetic acid 70% ethanol (FAA)⁵. Two hours after destaining, the solution was filtered and the absorbance of the root hair-free, coloured FAA solution was measured at 625 nm. In the standard curve for 0.02% toluidine blue O in FAA (figure 1) the abscissa represents an arbitrary scale of total root hair units (TRHU), which has been used here as an index of root hair growth.

For effective comparisons, the growth of intact root hairs was also studied both spectrophotometrically and by direct measurement using micrometers. The procedures for raising the seedlings and spectrophotometric estimation were similar to those described for studies on isolated root hairs. However, prior to staining with toluidine blue O the cotyledons were severed and the entire seedling axis was stained. In the study on direct measurement of root hairs, up to 100 root hairs were measured from five seedlings for each treatment and their average length calculated. Each of

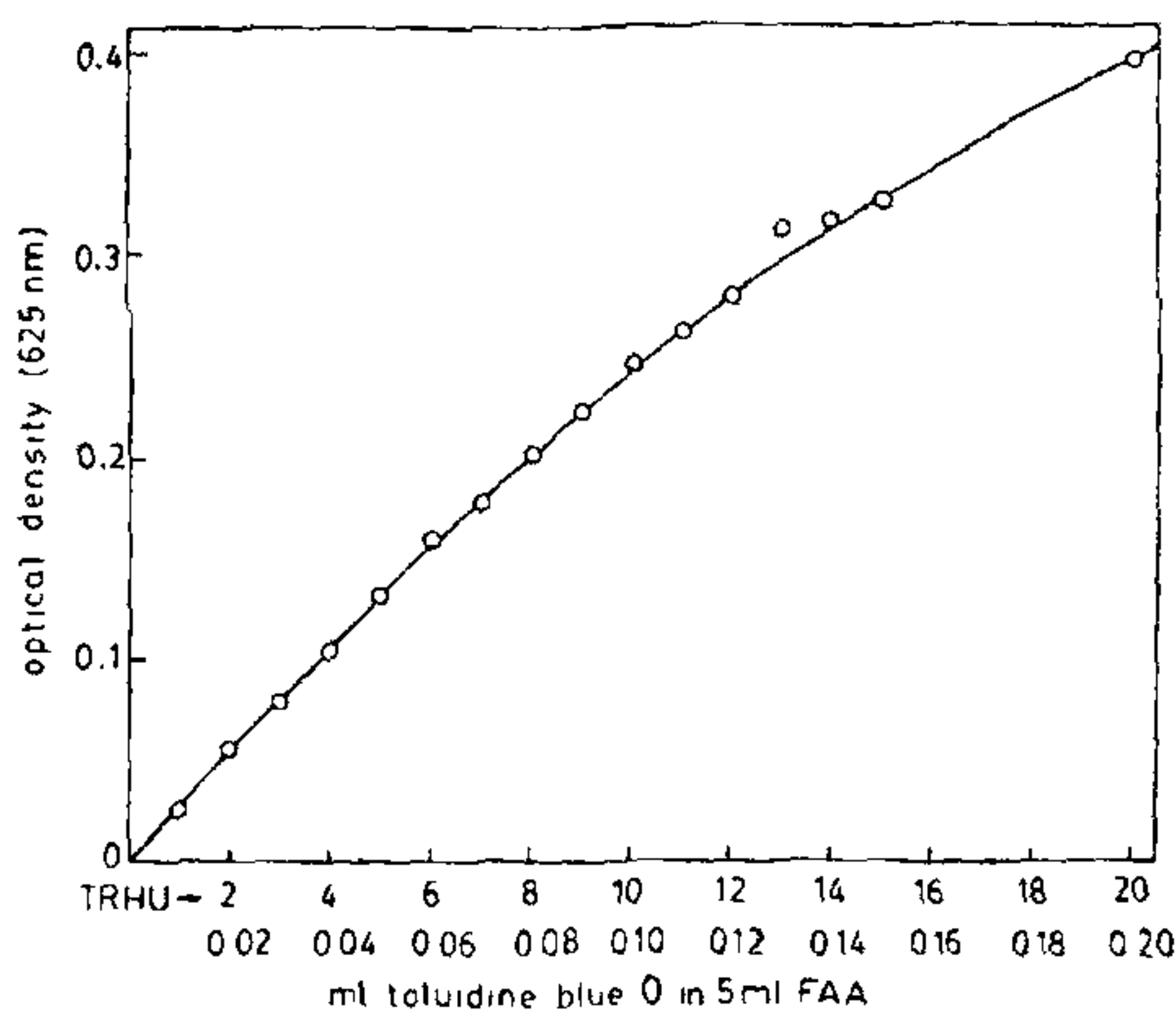


Figure 1. Standard curve for 0.02% toluidine blue O in FAA. Each value is an average of 5 determinations. The abscissa is an arbitrary scale of total root hair units (TRHU). The corresponding dilution series of toluidine blue O is also given.

the three studies, namely spectrophotometric analyses of root hair growth using (i) isolated root hairs and (ii) intact root hairs, and (iii) direct microscopic measurement of length of intact root hairs, was done eight times.

The TRHU in the control batches of seedlings was over three times that in treatment with 1.4×10^{-4} M dichlobenil in both isolated and intact root hairs (table 1). Intact root hairs invariably gave a higher TRHU than isolated root hairs. This was partly because the epidermis of the radicle also stained lightly with toluidine blue O, and the stained tiny root hairs near the tip of the radicle could not be isolated, and partly because the isolated root hairs probably lost some stain from their injured basal end. The per cent inhibition of TRHU generally increased with increasing concentrations of dichlobenil (table 1).

With increase in dichlobenil concentration the per cent inhibition of root hair elongation increased many-fold. Whereas the average root hair length in control was $2565 \mu\text{m}$, it decreased to $1140 \mu\text{m}$ in 1.4×10^{-4} M dichlobenil treatment.

In conclusion it can be stated that dichlobenil inhibited both the production of root hairs and their tip growth. These effects may be attributed to its properties as an inhibitor of cytokinesis^{7,8} and of cellulose synthesis². To my knowledge this is the first study on effects of dichlobenil on root hair growth. Like dichlobenil, cytochalasin B,

Table 1 Effects of dichlobenil on root hair growth in *Raphanus sativus*

Concentration of dichlobenil (M)	Spectrophotometric method				Direct method	
	Isolated root hairs		Intact root hairs		Length** (μm)	Percentage inhibition of elongation
	TRHU*	Percentage inhibition of TRHU	TRHU*	Percentage inhibition of TRHU		
0 (Control in distilled water)	6.7	0.00	11.5	0.00	2565	0.00
1×10^{-7}	6.2	7.46	13.0	(-)13.04	2472	3.62
5×10^{-6}	5.4	19.40	10.9	5.22	2129	16.99
5×10^{-5}	2.0	70.15	4.5	60.87	1308	49.01
1.4×10^{-4}	1.9	71.64	3.6	68.70	1140	55.55

* Mean of 8 determinations; ** Mean of 8 averages, each based on up to 100 determinations.

another inhibitor of cytokinesis also inhibited root hair growth in radish⁹. In cotton fibre growth, dichlobenil was *ca* 100 times as effective as coumarin in inhibiting cellulose synthesis³. The effects of dichlobenil are generally reversible^{3,7}. Whereas dichlobenil inhibits cell plate formation, it does not affect nuclear divisions^{7,8}. In this context experiments on cellularization in nuclear endosperms in angiosperms using dichlobenil and other cellulose synthesis inhibitors such as trehalose¹⁰ and isovitexin¹¹ should prove rewarding¹².

The author is grateful to Dr S. Nishimura, Division of Radiation Breeding, National Institute of Agrobiological Resources, Nakagun, Japan, for the gift of dichlobenil, to Professor N. S. Rangaswamy for guidance, and to Professor H. Y. Mohan Ram for his critical suggestions. Sincere thanks are also due to CSIR, New Delhi, for a fellowship.

26 December 1984

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ON MITOCHONDRIA OF *BUNOSTOMUM TRIGONOCEPHALUM* (RUD., 1808) RAILLIET, 1902

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MITOCHONDRIA are chiefly meant for oxidative phosphorylation. They have been reported to play an important role in the biology of nematodes also¹⁻³. Available literature⁴⁻¹⁸ reveals that much of the work done on the mitochondria of nematodes, deals with the pure biochemical or physiological aspects. The distribution of mitochondria in various tissues of the worms has not been studied so far at least in the case of *Bunostomum trigonocephalum*, a highly pathogenic hookworm prevalent in Meerut region in sheep and goat.

Large number of live worms were recovered from the intestines of sheep and goat obtained from a local abattoir. The worms thus obtained were washed thoroughly with double distilled water and fixed in Altmann's fluid¹⁹ or Zenker-formol fluid²⁰. Altmann's