Mode of Inhibitory Action of Clomeprop on Tuberization of *Cyperus serotinus**

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Abstract: Effect of clomeprop [2-(2, 4-dichloro-*m*-tolylloxy) propionanilide] on tuberization, RNA synthesis in the shoot, root and rhizome, and the morphology and anatomy of rhizome of *Cyperus serotinus* was studied. Clomeprop inhibited the tuberization concomitant with inducing rhizome abnormality regardless of the inhibitory activity on the shoot and root growth. In tuberization, increase in the cell number, cell size and starch deposit in the cells in initial swelling of the pith posterior to the leaf primordia in rhizome were inhibited by clomeprop. RNA synthesis in the rhizome was stimulated more remarkably than in other organs by the herbicide. It was suggested that clomeprop inhibited the tuberization of *C. serotinus* through its disturbing action on the RNA synthetic process followed by inhibition of the cell activity related to the morphological and anatomical changes associated with tuberization in the rhizome.

Key words: Clomeprop, *Cyperus serotinus*, tuberization, RNA synthesis, morphology and anatomy of rhizome

Introduction

Clomeprop [2-(2, 4-dichloro-*m*-tolylloxy)

propionanilide] has an auxin-like, highly selective herbicidal activity on broadleaved and cyperaceous weeds in paddy rice fields^{6,7)}. The herbicide is similar to naproanilide [2-(2-naphthoxy) propionanilide] in its chemical structure and herbicidal activity on the weeds^{2,8)}. Naproanilide was reported to inhibit the tuberization of the cyperaceous perennial weeds *Cyperus serotinus* and *Eleocharis kuroguwai*^{3,4)}. In our previous studies^{3,5)}, it was suggested that naproanilide disturbed the RNA synthetic process in the rhizome, thereby inhibiting the cell activity related to the morphological and anatomical changes associated with tuberization in the rhizome of the perennial weeds, and thus inhibiting the tuberization. However, it has not yet been made clear whether clomeprop has an inhibitory effect on the tuberization.

The present study was undertaken to investigate the effect of clomeprop on tuberization, morphology and anatomy of rhizome, and RNA synthesis of an individual organ of *C. serotinus*.

Materials and Methods

Plant materials

Sectioned tubers of *Cyperus serotinus* Rottb., each with a bud, were sprouted in an incubator at 30°C for 3 days, and then transplanted to a round plastic pot (113 mm in a diameter, 146 mm in height) filled with sand supplemented with 1 g of fertilizer (8-8-6) under a paddy field condition in an environment-controlled room. The room was maintained under a long-day condition (14 hr photoperiod provided by metal halide lamps at approximately 30 klux) at 28°C/23°C (illumina-

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tion/dark). Plants at the 4-leaf stage grown under these conditions for 1 month were used in all experiments.

Clomeprop treatment

One hundred ml of aqueous solution of clomeprop at various designated concentrations, containing 1% acetone and 0.1% Tween 20, was poured into the flooded water in each pot. Then the plants were either transferred to a short-day condition (8 hr photoperiod) at 28°C/23°C or allowed to remain longer in the long-day condition.

Effect on growth and tuberization

Harvesting was done 4 weeks after the treatment of clomeprop under the short-day conditions, and the number and dry weight of shoots, roots, rhizomes and tubers were determined. The onset of tuberization was observed at about 2 weeks under the short-day conditions.

Effect on morphology and anatomy of rhizome

The plants treated with clomeprop were harvested on the designated day periods of culture after the treatment, washed with water and the rhizomes were separated from the mother plants. The rhizome tips (about 1 cm) were fixed in formalin-acetic acid-ethylalcohol-water solution (1-10-2-7, v/v), dehydrated through an ethylalcohol series, and embedded in paraffin. They were sectioned at 10 μ m, mounted on slides, stained with Delafield's hematoxylin solution and eosine solution or with acid fuchsin solution, and then put under a microscope⁵⁾. Cell size, the number of cells and the starch deposit in cells located between vascular bundles in the pith about 1.5 mm below the base of leaf primordia in the rhizome were also measured.

Effect on radioactive uracil incorporation into RNA.

The plants were harvested at various time intervals after treatment with clomeprop and washed with water. Mother plants were used as the materials for the incorporation study. The basal parts of shoots (about 3 cm), the roots and

the rhizome except for those which had already developed into shoots or tubers were cut into small sections and incubated in 10 ml of incubation medium [0.01 M phosphate buffer (pH 6.0), 1% sucrose, 37 kBq uracil-2-¹⁴C (specific activity: 2.15 GBq/mM), and 8 ppm chloramphenicol]. After incubation for 6 hr at 30°C, fractionation and RNA estimation were done as previously reported³⁾. The rate of RNA synthesis was expressed by the amount of incorporated ¹⁴C per RNA content (dpm/OD₂₆₀).

All treatments were carried out with four replicates.

Results

Effect of clomeprop on growth and tuberization

The tuberization of *Cyperus serotinus* was inhibited by clomeprop to be almost completely at dosages of over 0.32 kg/ha, and severely at a dosage of 0.16 kg/ha (Table 1). The production of daughter shoot and rhizome was reduced by the herbicide at over 0.64 kg/ha. Abnormality such as twisting and antigeotropismal growth in the rhizomes, abnormal swelling in the basal parts of shoots and abnormal stumping in the top of newly developed roots was observed in plants treated with the herbicide at given dosages. The dry matter growth of shoot, root and rhizome was inhibited by clomeprop. However, the inhibitory effect was more remarkable on tuberization than on the growth of individual organs. The inhibitory and formative effects of clomeprop on the growth and tuberization of *C. serotinus* appeared similar to those of naproanilide³⁾, although the inhibitory activity of clomeprop on the growth of shoot and root and the production of daughter hills and rhizomes was more remarkable than those of naproanilide.

Effects on morphology and anatomy of rhizome

Since tuber initiation in *C. serotinus* was found to develop with the increase in the cell size, cell

Table 1. Effect of clomeprop on growth and tuberization of *C. serotinus*.

Clomeprop (kg/ha)	Shoot			Root		Rhizome			Tuber		
	No.	D.W. (mg)	Abnormality	D.W. (mg)	Abnormality	No.	D.W. (mg)	Abnormality	No.	D.W. (mg)	Abnormality
0	100 (4.1)	100 (1500)		100 (77)		100 (17.3)	100 (233)		100 (13.0)	100 (102)	
0.16	117	92	*	71	*	58	81	*	10	3	
0.32	107	102	*	101	*	56	73	**	2	1	
0.64	72	70	**	49	*	43	45	**	2	1	
1.60	45	54	**	42	**	33	53	**	0	0	

Note : a) Data are presented as percent of the control except for values in parentheses, which indicate the number and dry weight of shoots, roots, rhizomes or tubers per plant.

b) The number of asterisks indicates the degree of abnormality observed at harvest ; none indicates no abnormality.

number and starch deposit in cells located in the pith posterior to the leaf primordia in the rhizome⁵⁾, the effects of clomeprop on these changes associated with tuberization were determined at the dosage of 0.64 kg/ha (Fig. 1). An inhibition of pith thickening by the herbicide under the short-day condition was notable after 7 days, and the rate of this inhibition was further enhanced as a function of time. The increase in the cell number was inhibited at 7 days and the inhibition degree became more conspicuous with the passing of time after treatment. The increase in cell length both radially and longitudinally were inhibited by clomeprop : 3 days radially and 7 days longitudinally, respectively. The inhibition became more conspicuous with time after treatment. However, the inhibition of cell elongation radially was much more remarkable than that longitudinally at any given time. Clomeprop thus inhibited not only cell division but also cell enlargement, thereby blocking the pith thickening in rhizome under the shortday condition (tuberizing condition). The starch deposit in the cells located in the pith of rhizome was completely inhibited for up to 2 weeks after the treatment.

It was demonstrated that the morphological and anatomical changes associated with tuberization in the rhizome were remarkably inhibited by clomeprop to a degree similar to those of untreated

control under the long-day condition (non-tuberizing condition), especially in the radial cell elongation and in the starch deposit in cells.

Effect of clomeprop on RNA synthesis

The effect of clomeprop (0.64 kg/ha) on RNA synthesis in each organ was studied at various intervals following the treatment (Fig. 2). No significant effect on RNA synthesis was found in any organ one day after the treatment. At 3 days, RNA synthesis in the rhizome was apparently stimulated, and was further enhanced 7 days after the treatment. In the shoots, a stimulation of RNA synthesis was notable 7 days but not 3 days after the treatment, while in the root there was scarcely any effect at either of these times. The results demonstrated that RNA synthesis was more remarkably stimulated in rhizome than in shoot and root by clomeprop, and this occurred in a dosage dependent manner at any given time after the treatment (Fig. 3).

Rhizome was thus the most sensitive of all organs to clomeprop in terms of RNA synthesis stimulation, similar to that of naproanilide³⁾.

Discussion

This study demonstrated that clomeprop inhibited the tuberization of *C. serotinus* with abnormal growth in the rhizome, as true with naproanilide³⁾; the inhibitory activities of clome-

Fig. 1. Effect of clomeprop on morphological and anatomical changes in the pith of rhizome of *C. serotinus*¹⁾.

—●— Untreated control under short-day condition.

—▲— Untreated control under long-day condition.

—○— Clomeprop treatment (0.64 kg/ha) under short-day condition.

Fig. 2. Effect of clomeprop (0.64 kg/ha) on RNA synthesis in shoot, root and rhizome of *C. serotinus*.

Fig. 3. Effect of various dosage rates of clomeprop on RNA synthesis in rhizome of *C. serotinus*.

prop on the dry matter growth of shoot and root and the daughter shoot and rhizome production were, however, stronger than those of naproanilide. Clomeprop affected the cell division, cell enlargement and starch deposit in the pith of rhizome, resulting in inhibition of the morphological and anatomical changes associated with tuberization. Rhizome was the most sensitive of all organs to clomeprop in terms of the disturbance of the RNA synthesis process. The effects of clomeprop on RNA synthesis and the morphology and anatomy of rhizome were also similar to those of naproanilide⁵. It is thus suggested that disturbance of the RNA synthetic

process by clomeprop in rhizome might induce the inhibition of morphological and anatomical changes associated with tuberization in a similar manner to naproanilide^{3,5}. Consequently, it is proposed that both herbicides, which have an auxin-like activity, inhibit in a similar mode the tuberization of *C. serotinus* through biochemical and morphological action on the developmental function of rhizome into tuber. The tuberization of perennial weed has suggested to be regulated by plant hormones through their action on the developmental function of rhizome into tuber^{1,4}. It thus seems reasonable to assume that clomeprop and naproanilide inhibit tuberization by the auxin-like activity on RNA metabolism in rhizome, and that this is followed by inhibition of the morphological and anatomical changes associated with tuberization in rhizome of *C. serotinus*.

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ミズガヤツリの塊茎形成、根茎の内部形態変化および RNA 生合成に対するクロメプロップの作用

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摘 要

クロメプロップ [2-(2,4-dichloro-*m*-tolylloxy) propionanilide] のオーキシシン活性に着目し、ミズガヤツリ (*Cyperus serotinus* Rottb.) の塊茎形成に対する作用を調べた。また、塊茎形成に対する作用と根茎の内部形態変化および各器官の生育、RNA 生合成に対する作用との関連性についても検討した。

1) 塊茎形成に対する本剤の阻害作用は強く、いずれの施用量においても強い阻害が認められた (第1表)。しかし、塊茎形成阻害作用に比べ、茎葉、根および根茎の生育に対する抑制作用は弱かった。また、これら各器官には、いずれも奇形が観察されたが、発現程度は根茎において顕著な傾向がみられた。

2) 本剤は、根茎先端部に存在する葉原基の下方

部に位置する髓の内部形態に強く作用し、本来ならば塊茎形成に伴って増加する細胞の分裂、伸長、肥大、細胞内でのデンプン蓄積および髓の肥大のいずれもが抑制された (第1図)。なかでも、根茎の肥大方向への細胞伸長とデンプン蓄積が特に強く抑制された。

3) 各器官の RNA 生合成は、処理1日後ではほとんど本剤の影響を受けなかった。根茎では、処理3日後に促進がみられ、7日後にはさらに顕著に促進された (第2図)。しかし、茎葉では7日後になってはじめて促進がみられた。また、根では処理後の日数にかかわらずほとんど影響を受けなかった。根茎の RNA 生合成は本剤の処理量が多いほど顕著に促進された (第3図)。

以上の結果から、クロメプロップはミズガヤツリの塊茎形成を強く阻害することが明らかになった。また、塊茎形成阻害作用は、根茎の RNA 生合成系が攪乱されることにより塊茎形成に伴う根茎の内部形態の変化が阻害されて発現するものと想定される。

キーワード: クロメプロップ, ミズガヤツリ, 塊茎形成, RNA 生合成, 根茎の内部形態

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