Phyton (Austria)	**Vol. 25**	Fasc. 2	233–240	30. 11. 1985

The Effects of Dibrom on Shoot Esterase and Certain Stages of Development in the Genus *Taeniatherum (Poaceae)*

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With 2 Figures (1 Plate)

Received July 28, 1984

Key words: *Gramineae, Poaceae, Taeniatherum.* – Esterase patterns, phylogenetic relationships, organophosphorus inhibitor

Summary

SYMEONIDIS L. A. 1985. The effect of dibrom on shoot esterase and certain stages of development in the genus *Taeniatherum.* – Phyton (Austria) 25 (2): 233–240, with 2 figures (1 plate). – English with German summary.

The esterase patterns of two native greek forms of *Taeniatherum caput-medusae* and their behaviour, in vitro, to dibrom and eserine as well as the effects of different dibrom (trade name Naled) concentrations on seed germination, shoot and root growth were studied. It was found that dibrom is an essential inhibitor for esterases, but there was no inhibition with eserine even at a concentration of 10^{-3}M suggesting that the studied esterases may be carboxylesterases. Seed germination shot and root growth showed a negative correlation to increasing concentration of dibrom with a sharp decline following the 10^{-4}M concentration.

The behaviour of both forms to dibrom was similar, a fact that in connection with the large number of common bands of their esterase patterns underline their close relationship.

Zusammenfassung

SYMEONIDIS L. A. 1985. Effekte von Dibrom auf die Esterase in Sprossen und einige Entwicklungsstadien von *Taeniatherum (Poaceae).* – Phyton (Austria) 25 (2): 233–240, mit 2 Figuren (1 Tafel). – Englisch mit deutscher Zusammenfassung.

Die Esterase-Muster von zwei in Griechenland einheimischen Formen von *Taeniatherum caput-medusae* und ihr in vitro – Verhalten gegenüber Dibrom (Han-

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delsname Naled) und Eserin, ferner die Wirkung von Dibrom in verschiedenen Konzentrationen auf Samenkeimung, Sproß- und Wurzelwachstum wurden untersucht. Dibrom erwies sich als Inhibitor für Esterasen, hingegen hemmten selbst 10^{-3} M Eserin nicht, was nahelegt, daß die untersuchten Esterasen Karboxylesterasen sein dürften. Samenkeimung, Sproß- und Wurzelwachstum zeigten zu ansteigenden Dibrom-Konzentrationen negative Korrelation mit einem steilen Abfall über 10^{-4} M. Die beiden Formen verhalten sich gegenüber Dibrom ähnlich, was mit der großen Zahl gemeinsamer Esterase-Banden zusammenhängt und ihre nahe Verwandtschaft unterstreicht. (Editor transl.)

Introduction

The genus *Taeniatherum* belongs to the family *Gramineae (Poaceae)* and according to HUMPHRIES 1980, it comprises one annual diploid species (2 n = 14) and one variant from Crym, while NAKAI & SAKAMOTO 1977 distinguish two annual diploid species.

Organophosphorus compounds and eserine have been used by many workers in 1) classifying esterases (ALDRIDGE 1953 a, b, BERGMANN & al. 1957, AUGUSTINSSON 1961, MONTGOMERY & al. 1968, VEERABHADRAPPA & MONTGOMERY 1971, KARATAGLIS & TSEKOS 1975, MACDONALD & BREWBAKER 1975 and 2) in studying the effect of organophosphorus poisons on mitosis in plants (AMER & FARAH 1974) and their toxic action on animals and plants (VAN ASPEREN 1960, O'BRIEN 1967, BULL 1972, LIU & al. 1981, BONETA-GARCIA 1982).

In the work reported in this paper an attempt was made to evaluate the effects, in vitro, of different levels of dibrom (trade name Naled) and eserine on *Taeniatherum* esterase isoenzymes, as well as the effect of dibrom on seed germination, shoot and root growth.

Materials and Methods

Two native forms of the greek flora belonging to *Taeniatherum caput-medusae* NEVSKI and coexisting in the same populations, were studied: *Taeniatherum caput-medusae* forma I (2 n = 14) and *T. caput-medusae* forma II with the same chromosome number but with seeds and awns a little smaller.

For the electrophoresis and for checking the effects of dibrom on seed germination, shoot and root growth, seeds of more than 30 plants collected randomly from each population from various regions (see Table 1) of Northern Greece were used.

The procedures followed for seed germination, electrophoresis (shoots of 8-d-old seedling and α-naphthyl acetate as substrate were used) and staining of esterases were described in previous papers (SYMEONIDIS & al. 1979, SYMEONIDIS & TSEKOS 1984).

In order to estimate the effects of a grading concentration of two selective inhibitors on esterases, namely dibrom (dimethyl-1,2-dibromo-

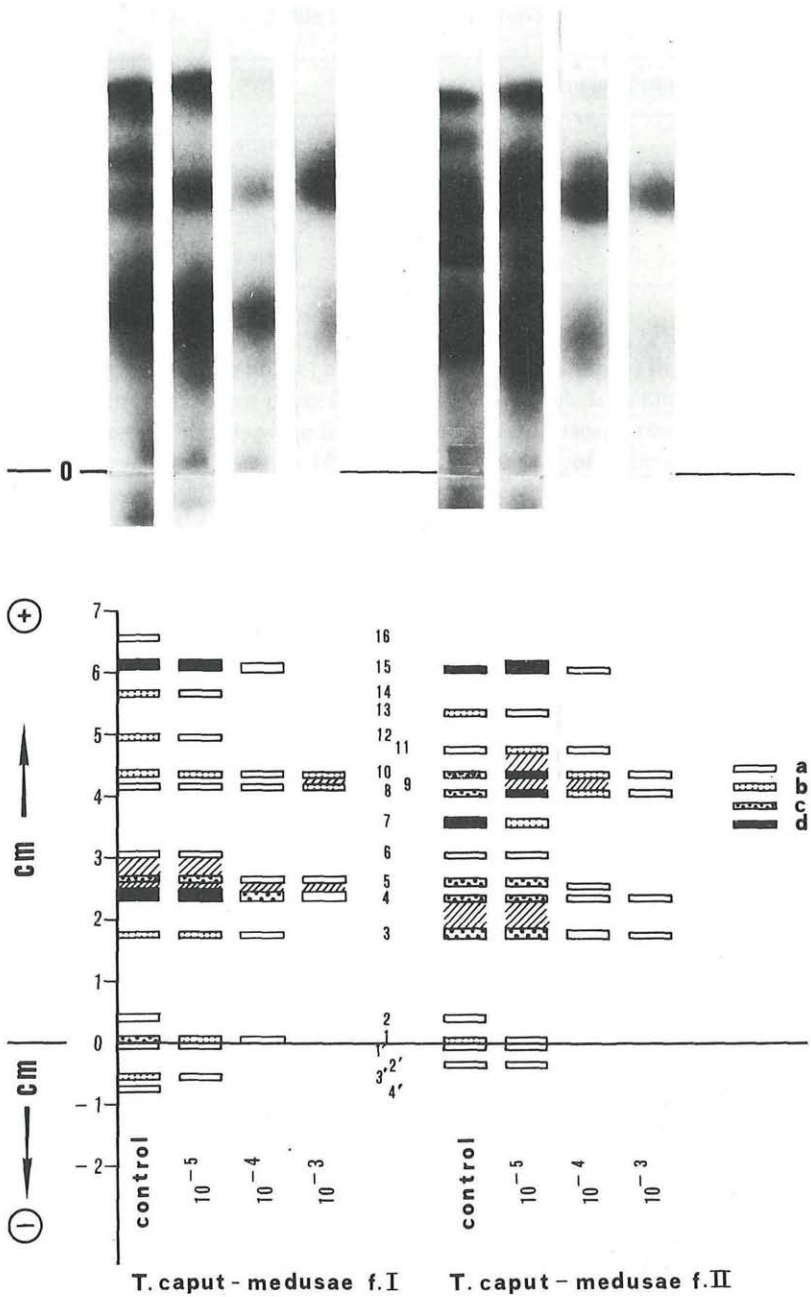

Fig. 1. Effects of dibrom on shoot esterase patterns of *T. caput-medusae* forma I and forma II. Photograph and corresponding schematic representation. On the right: key to shading in order of increasing band intensities.

Table 1. Sources of materials

Localities	Number of plants
Mandra (Xanthi)	31
Mavrovouni (Edessa)	29
Moudania (Halkidiki)	30
Mount Hortiatis	43
Sostis (Komotini)	30

2,2-dichloroethyl phosphate) and eserine (eserine sulfate) in vitro, we have employed the following procedure (see KARATAGLIS & TSEKOS 1975). After electrophoresis the gels were incubated for 30 minutes at room temperature in some concentrations (0, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} M) of dibrom and (0, 10^{-4}, 10^{-3} M) of eserine. The inhibitors were removed, the gels rinsed well with running distilled water and the above mentioned method was used for staining the esterases.

In addition the effects of the concentrations 0, 10^{-5}, 10^{-4}, – 10^{-3}, 5×10^{-3}, 10^{-2} M of dibrom on seed germination, shoot and root growth were studied. The seeds were germinated in petri dishes placed in a thermostat at about 21.5±1° C in the dark with and without the appropriate levels of inhibitor. There were two replications of each concentration with thirty plants in each replication. After 8 days the number of germinated seeds, as well as the length of shoots and roots in each concentration were measured.

Results

1. Shoot esterase patterns and their behaviour to dibrom and eserine.

Two basic esterase patterns were found in all studied populations. One is referred to *T. caput-medusae* forma I with 15 bands (12 anodal and 3 cathodal) and one to *T. caput-medusae* forma II with 14 bands (12 anodal and 2 cathodal) (Fig. 1). There was a large number of bands of high electrophoretic mobility in both materials and nine bands in common.

Compared to the controls a concentration of 10^{-5} M dibrom has resulted in eliminating the 4, 2 and 16 bands of *T. caput-medusae* forma I and band 2 of *T. caput-medusae* forma II, and in changing the intensities of some bands (Fig. 1).

As the concentration of the dibrom increases, the number and the intensities of bands decrease. So in the 10^{-4} M dibrom concentration, 7 bands have remained in *T. caput-medusae* forma I (1, 3, 4, 5, 9, 10 & 15) and seven in *T. caput-medusae* forma II (3, 4, 5, 8, 10, 11, & 15) (Fig. 1).

In concentration of 10^{-3} M dibrom the number of bands is restricted to 4 (4, 5, 9 & 10) in *T. caput-medusae* forma I and 4 (3, 4, 8 & 10) in *T. caput-*

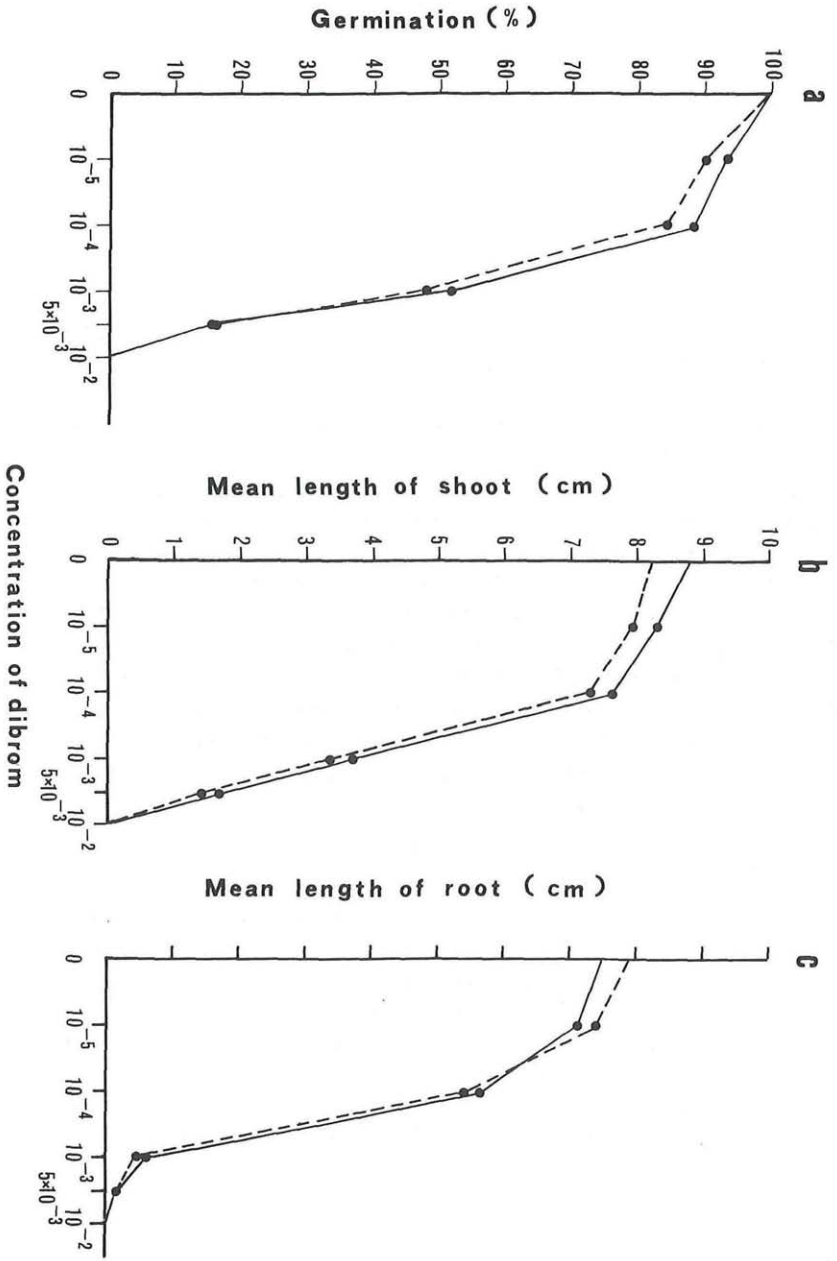

Fig. 2. Diagrams showing the effects of dibrom on a) seed germination % b) mean length of shoot (cm) and c) mean length of root (cm) for *T. caput-medusae* forma I (———), and *T. caput-medusae* forma II (– – – –).

medusae forma II. The 10^{-2} M dibrom concentration fully inhibits all bands in both materials (Fig. 1).

Despite the fact that eserine has been reported to be inhibiting the esterase activity it seemed to very little affect the enzymatic activity of esterases at concentrations 10^{-4} M and 10^{-3} M.

2. Effects of dibrom on seed germination, shoot and root growth.

Fig. 2 shows the effects of a grading concentration of dibrom on seed germination, shoot and root growth in both materials studied. If we consider the germination of controls as 100% then we can see that as the dibrom concentration increases the rates of germination decline linearly down to the 10^{-4} M concentration. Note that after the above concentration the rate of germination reduces sharply.

Compared to the controls the shoot growth is significantly inhibited at 10^{-4} M dibrom and rapidly declines with increased dibrom at 10^{-3} M. Shoot growth is reduced very much at a concentration of 5×10^{-3} M (see Fig. 2 b).

A significant decline in root lengtt relative to the controls occurs in both materials with increasing dibrom concentration at 10^{-4} M (Fig. 2 c). The marked inhibitory effect of dibrom at the concentration of 10^{-3} M and 5×10^{-3} M on root growth is shown in Fig. 2 c.

Discussion

According to BOULTER & al. 1966, bands from different taxa but with the same mobility are likely to be produced by genes common to both taxa, while bands that migrate differently are likely to be controlled by different genes or different alleles of a locus.

In this respect the comparison of band positions and zymograms in the studied materials allow us to distinguish two forms, namely, *T. caput-medusae* forma I and *T. caput-medusae* forma II. Fig. 1 demonstrates that the two forms have 9 bands in common out of a total of 15 in *T. caput-medusae* forma I and of 14 in *T. caput-medusae* forma II displaying their close relationship (see also SYMEONIDIS & TSEKOS 1984).

Fig. 1 shows that there is a gradual decrease of enzymic activity as the concentration of the inhibitor increases. In a concentration of 10^{-2} M the enzymic activity is completely inhibited, showing the sensitivity of the studied esterases to dibrom. This happens to be in agreement with the finding that organophosphorus compounds are potent enzymic inhibitors in animals and plants and were used as pesticides, insecticides and herbicides (VAN ASPEREN 1960, O'BRIEN 1967, BULL 1972, LIU & al. 1981, BONETA-GARCIA 1982).

This inhibition of enzyme may be due to the reaction of dibrom and enzyme resulting in a complex which reacts to give a phosphorylated enzyme. The site phosphorylated is the esteratic site in the active centre.

This is thereby blocked, so that the enzyme is unable to hydrolyse its normal substrate, i. e. it is inhibited (MYERS & al. 1957, HEATH 1961, O'BRIEN 1967).

Inhibition by organophosphorus compounds has been the basis for the classification of esterases (ALDRIDGE 1953 a, b, BERGMANN & al. 1957, AUGUSTINSSON 1961, MONTGOMERY & al. 1968, MACDONALD & BREWBAKER 1975). Cholinesterases are distinguishable from other ester hydrolases by their sensitivity to eserine. ALDRIDGE 1953 a, b and BERGMANN & al. 1957 further proposed that arylesterases and acetylesterases could be differentiated from the carboxylesterases by their resistance to organophosphates. As mentioned earlier the esterases studied in both materials are resistant to eserine that is often used to differentiate the cholinesterases, which are specifically inhibited at concentrations that do not affect other esterases (for example 10^{-3} M) (MACDONALD & BREWBAKER 1975).

From the above we can suppose that the esterases studied may be carboxylesterases because of their sensitivity to organophosphorus compounds and their resistance to eserine.

When the inhibitor dibrom acts during the germination it results in a gradual inhibition of seed germination reaching the 10^{-4} M dibrom concentration beyond which seed germination is sharply inhibited (Fig. 2 a).

The action of the inhibitor during germination has also resulted in shoot and root growth. The mean length of shoot and root reduces gradually as the dibrom concentration increases up to the concentration of 10^{-4} M beyond which, as in the case of seed germination the shoot and root length reduces rapidly.

In *T. caput-medusae* forma I the reduction of the shoot length at a concentration of 10^{-4} M is 89.4%, while that of the root is 74.6% of the controls. In the 10^{-3} M concentration the shoot length is restricted to 43.5% and the root length to 7.5% of the controls.

Almost the same is valid for *T. caput-medusae* forma II. Here the mean length of the shoot at 10^{-4} M and 10^{-3} M concentrations is 89.0% and 41.5% respectively whereas of the root 68.3% and 5.7% of the mean length of the controls. As shown in Fig. 2 b, c the most conspicuous results are manifested in the root. This is because the root is the organ to be in direct contact with the organophosphorus inhibitor that effects a great decrease in the mitotic index of root-tip cells (AMER & FARAH 1974) and consequently a vigorous inhibition of its development and of the root surface of water uptake.

On the basis of our electrophoretic results the behaviour of the studied esterase patterns to dibrom and eserine and the effects of dibrom when acting during germination, we may conclude that 1) the esterases investigated may be carboxylesterases because of their sensitivity to organophosphorus compound dibrom and their resistance to eserine, and 2) the two studied forms are closely related. This comes from the large number of common bands they have and from the close similarity of their reaction to dibrom and eserine.

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Recensio

SENGER H(orst) (Ed.) 1984. Blue Light Effects in Biological Systems. Proceedings in Life Sciences. – Gr.-8°, XVI + 538 Seiten mit 298 Abbildungen, Leinen gebunden. – Springer Verlag Berlin–Heidelberg–New York–Tokyo. – DM 142,–. – ISBN 3-540-13462-X.

Vier Jahre nach dem ersten Internationalen Kongreß über Wirkungen von Blaulicht auf Pflanzen und Mikroorganismen (vgl. Rezension SENGER (ed.) 1981, Phyton 24: 393) fand im Juli 1984 wieder in Marburg/L. unter der gleichen Organisation ein weiterer Kongreß zu diesem Thema statt. Es ist erfreulich, daß schon nach kurzer Zeit die damals gehaltenen Vorträge gesammelt vorliegen. Sie beschäftigen sich fast ausschließlich mit den seither erzielten Fortschritten. Es ist im Rahmen einer kurzen Rezension unmöglich, alle 57 Originalbeiträge auch nur zu erwähnen. In einem einleitenden Abschnitt hebt KRITSKY das hohe Alter der Blaulichtrezeptoren hervor, sie sind wohl bereits vor 3,5 Milliarden Jahren, also noch unter anaeroben Bedingungen entstanden. Als Pigmente kommen vor allem Flavoproteine und Carotine in Betracht, doch sollten auch andere Pigmente, wie Tetrapyrrole, nicht übersehen werden (SONG). Für derartige blaulichtempfindliche Pigmente hat sich, zunächst im Laboratoriumsjargon, die Bezeichnung „Cryptochrome" gebildet; sie wurde zwar auf dem ersten „Blaulichtkongreß" abgelehnt, auf dem zweiten jedoch wurde sie, wie SENGER in einer kurzen terminologischen Notiz einräumt, vorläufig, d. h. bis zum Vorliegen genauerer Definitionen, für photosensitive Pigmente, die im Bereich unterhalb 520 nm bis ins UV absorbieren, akzeptiert. Ein Block von Beiträgen befaßt sich mit Blaulichtrezeptoren in verschiedenen Pflanzengruppen, ein weiterer mit deren Natur und Eigenschaften (Carotin – Flavin – Problem, membrangebundene Flavine), weitere Blöcke mit den Primärreaktionen und der Signalübermittlung (9 Beiträge), zwei weitere mit der Wirkung von Blaulicht auf Enzymregulation, Kohlenhydratstoffwechsel sowie mit der Biosynthese in Frage kommender Pigmente. Erwartungsgemäß nehmen Beiträge über Wachstum und Entwicklung sowie Bewegungen (Zellkulturen, Algenchloroplasten und deren Bewegungen, Phototaxis von *Physarum polycephalum*, Phototropismus von Maiskeimlingen, Stomatabewegungen) breiten Raum ein (9 Beiträge. Ein Index der erwähnten Organismen sowie ein Sachverzeichnis schließen den Inhalt des aktuellen Bandes ausreichend auf.

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