Studies on a Herbicide, Benthocarb

IV. Histological effects of benthocarb on barnyardgrass

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Benthocarb [S-4 (chlorobenzyl) N,N-diethylthiolcarbamate] is highly effective against barnyardgrass (*Echinochloa crusgalli* L. Beauv. var *oryzichola*)^{2,3,8,11)}. Though it is a selective, non-hormonal herbicide, it produces formative effects on barnyardgrass and other susceptible plants. In barnyardgrass, the herbicide alters the marginal and upward growth of the leaves but no histological basis of these alterations, except that of tubular leaf, and that too, with a difference of opinion, has been described^{15,16)}. The effect of the herbicide on the shoot apex of barnyardgrass is also in confusion. Shigematsu and Kimura¹⁵⁾ reported that the herbicide had little effect on shoot apex of injured seedlings, but Shibayama and Worley¹⁶⁾ reported that the herbicide caused necrosis of the cells in the shoot apex.

To re-examine these points of contradiction and to describe the morphological and histological aspects of the mode of action of benthocarb, we have been investigating the effects of this herbicide on barnyardgrass. In the previous papers^{12,13)}, we reported the effect of the herbicide on the gross morphology and the morphogenesis of the plant at different growth stages. In this paper, we shall present the histological effects of the herbicide on barnyardgrass.

Materials and Methods

Barnyardgrass plants were raised in the Wagner's pot or plastic planters in the glass house under natural day-length and temperature and treated with different amounts of benthocarb as reported previously¹²⁾. The plants were observed very carefully for any morphological change due to benthocarb treatment. For histological observations, the desired materials were sampled

and fixed with FAA. The fixed materials were dehydrated in an ethanol-normal butyl series, embedded in paraplast and sectioned at 8 to 10 μ m by a rotary microtome. The sections were stained with Delafield's Hematoxylin or ferric chloride-safranin-fastgreen. When the latter tripple stain was used the sections were mordanted by tannic acid before staining. Observations have been illustrated by photograph.

Results

The histological effects of benthocarb on barnyardgrass have been presented below.

1. *Swelling at the base of the coleoptile* (Fig. 1a & b) There was a remarkable swelling at the base of the coleoptile. This swelling was not due to the cell proliferation at that region but for an outward folding of the coleoptile all around the base. In such a seedling the divergence angle of the vascular bundle of the coleoptile with the main axis was much greater than that in the untreated seedling.

2. *Sinuation of the leaf* (Fig. 2) In a longitudinal section through the tubular sheath of the first leaf of a seedling, the inner leaf was seen to be sinuated. In such a leaf the linear rows of the cells were buckled; the inner and outer walls of the individual cell elongated differentially throughout the length of the leaf.

3. *Undulation of the leaf surface* (Fig. 3a & b) The adaxial surface of the inner leaf became undulated. In such a leaf, a differential increase in the cell tiers and differential elongation of the cells in radial or tangential direction at the adaxial side of the leaf was observed.

4. *Distorted upward growth of the leaf* (Fig. 4) On longitudinal sections of a seedling,

the vascular bundles of the inner leaf was seen in transverse plane. This was perhaps due to abnormal orientation of the inner leaf due to distorted upward growth.

5. *Increase of lateral appendages (Fig. 5a & b)* The number of lateral appendages were always more in the treated seedlings than the untreated ones. Lateral appendages were also observed around the shoot apices in the treated seedlings. These appendages were the primary, secondary, and probably, the tertiary tillers that had been stimulated by the herbicide to initiate but the further growth of which were checked by the inhibiting effects of the herbicide.

6. *Necrosis of the leaf tissues (Fig. 6)* The cells probably, at the region of the lamina-joint, of the young leaf were necrosed. The cells in the outer, older leaves, however, collapsed due to extensive vacuolation and enlargement.

7. *Elongation of shoot apex (Fig. 7a & c)* The cells in the tunica and corpus elongated longitudinally and as a result, the shoot apex, as a whole, elongated.

8. *Induction of periclinal divisions at abnormal sites on the tunica* In some individuals the periclinal divisions in tunica were observed at comparatively higher level towards the tip of the shoot apex (Fig. 7b). Periclinal division was also observed at the tip of the shoot apex (Fig. 7c).

9. *Induction of tubular leaf* Tubular leaf was the most frequently observed aberration in the barnyardgrass seedlings treated with benthocarb. The first one or two leaves initiating after the treatment turned into tubular. The morphology of a tubular leaf has been presented in a previous paper¹²⁾. Histologically, the marginal wings of a tubular leaf was seen to be fused continuously or discontinuously up to a certain length of the leaf. In some individuals, the margins at the sheath became fused and the margins at the lamina region remained separated (Fig. 8a, b, c & d). In other individuals the margins towards the bases of the sheath and lamina remained fused, while the upper parts of the sheath and lamina remained separated. In others the margins remained fused throughout the

length of the leaf except at the tip (Fig. 9).

10. *Adnation of two leaves (Fig. 10)* In some individuals a part of the two successive leaves remained adnated longitudinally. This was due to the fusion of a part of the adaxial side of the outer leaf to the abaxial side of the inner leaf. And, as a result, the epidermal cells at the fused portion of the two leaves could not be seen. This fusion was caused at the primordial stage of the leaves.

11. *Change of overlapping manner of the margins (Fig. 11a & b)* In some treated and untreated individuals, the overlapping manner of the marginal wings being similar in the first leaf was reversed in the 2nd, 3rd, and 4th leaves.

12. *Inflexion and deflexion of the marginal wings* In some individuals one of the margins of a leaf inflexed very sharply and the other only a little and both the margins continued growth (Fig. 12).

In some seedlings both the margins met end to end at first and thereafter, deflexed slightly and grew upward (Fig. 13).

In others, one of the margins of a leaf inflexed and the other deflexed. The inflexed margin grew inward and the deflexed margin grew upward and thus, the marginal wings of a single leaf geared with each other (Figs. 14 & 15).

13. *Truncation of the margins (Fig. 16)* In some leaves the margins, instead of overlapping, became truncate, met end to end and remained touched to each other but did not fuse.

14. *Sinuation of the marginal wings (Fig. 17)* In some seedlings one of the overlapped marginal wings of a leaf sinuated very remarkably and the other only a little.

15. *Tightly overlapping of the margins (Fig. 18)* The leaf margins in most of the treated individuals did not overlap loosely as those in the untreated seedlings. Instead, they remained adpressed over each other very tightly and exhibited a compact structure. Abnormal radial enlargement of the cells at the adaxial side of the marginal wings was observed in such individuals.

16. *Extensive vacuolation and enlargement of cells (Fig. 9)* In the treated seedlings the

Fig. 1. Longitudinal sections of untreated (1a) and treated (1b) 5 day's old barnyardgrass seedlings. Note the outward folding of the coleoptile at the base in the treated seedling. Also note that the diverging angle of the coleoptilar vascular bundle (indicated by arrow) is much greater in the treated seedling than in the untreated one ($\times 43$ approx.).

Fig. 2. Longitudinal section through the tubular sheath of the first leaf of a seedling showing the situation of the inner leaf ($\times 80$ approx.).

Fig. 3. Longitudinal section 10 day's old treated seedling showing the undulated adaxial surface of a inner leaf (3a, $\times 30$ approx.). 3b is the enlarged view ($\times 160$ approx.) of the portion indicated by in 3a.

Fig. 4. Longitudinal section of a 15 days' old treated seedling showing the vascular bundles of an inner leaf in transverse plane. Arrows indicate the vascular bundles ($\times 160$ approx.).

Fig. 5. Longitudinal sections of 10 days' old untreated (5a) and treated (5b) seedlings. Note several lateral appendages (indicated by arrows) in the treated seedling ($\times 105$ approx.).

Fig. 6. Longitudinal section of a 15 days' old treated seedling showing necrosed area (indicated by arrow) of leaves ($\times 80$ approx.).

Fig. 7. Longitudinal sections of shoot apices of 10 days' old seedlings. 7a is of the untreated, and 7b and 7c are of the treated seedlings. In 7b note the periclinal divisions in tunica

(indicated by arrows) near the tip of the shoot apex. In 7c note that there is a periclinal division at the shoot apex and that the apex is elongated ($\times 420$ approx.).

Fig. 8. Serial transections of a 10 days' old treated seedling. Note the marginal wings of a leaf are fused forming a tubular structure in the sections below the shoot apex (8 a, 8 b, 8 c) and are separated in the section (8c) through the shoot apex. ($\times 120$ approx.).

Fig. 9. Transection through the lamina region of a tubular leaf. Note the cells in the adaxial epidermis are enlarged radially ($\times 80$ approx.).

Fig. 10. Transection of a 15 days' old seedling which was treated at 10-day stage showing the adnation of two successive leaves. Note that the portions shown in between two arrows are fused and no separate epidermis is seen ($\times 80$ approx.).

Fig. 11. Transections of 10 days' old seedlings. 11a is of untreated and 11b is of treated seedling. Note that the overlapping manner of the first leaf (indicated by arrow) are similar in both the sections but that of other leaves are reversed ($\times 80$ approx.).

Fig. 12. Transection of a treated seedling through the leaf. Note one of the marginal wings is sharply inflexed (indicated by arrow, $\times 310$ approx.).

cells in the injured leaves vacuolated extensively and enlarged tremendously. The cells in the abaxial side enlarged tangentially while those in the adaxial side enlarged radially. The chloroplast in the vacuolating cells arranged near the cell walls.

17. *Abnormal growth of the vascular bundle* (Fig. 18) In transverse section, in some affected leaves the vascular bundles were observed in irregular shapes and the tracheary and sieve elements were observed in longitudinal plane. All other cells, however, were in transverse plane. This might have been due to the abnormal growth of the vascular bundle.

18. *Inhibition of leaf production* The number of leaf in a 15 days' old untreated seedling was always 8 but that in 2.0 kg/ha a.i. treated seedling was always 5.

Discussion

The induction of tubular leaf, and change of overlapping manner, tightly overlapping, inflexion, deflexion, sinuation, and truncation of margins are the most note-worthy histological effects of benthicarb on barnyardgrass plants. These aberrations were observed most frequently in this study and were caused due to abnormalities induced by the herbicide in the marginal growth of the developing leaves. We observed that the leaf which initiated at the time of treatment became tubular. To form a tubular leaf, we consider that, during the marginal growth of the leaf primordium the two

marginal wings of a single leaf primordium did not overlap normally but, instead, they met end to end and fused, and the primordium grew with the margins joined together. Being tubular when a leaf primordium continued its growth, sometimes one of the margins resumed the normal growth and formed one wing as shown in Fig. 9. Tubular leaf of the similar type are also reported to be produced by hormone herbicides in susceptible plants^{5,9,100}. Thus, on the structure of tubular leaf in barnyardgrass due to benthicarb treatment, we agree to Shigematsu and Kimura¹⁵³, and disagree to Shibayama and Worley¹⁶⁹. Except the tubulation of the leaf and the truncation of the margins, all other aberrations, as stated above, were newly found out by us in the benthicarb treated plants and no one has yet reported such effects of benthicarb or other herbicides on the marginal growth of leaves of susceptible plants. These aberrations, we consider that, were associated with the differential rate of cell division and/or cell elongation in the marginal meristem and induced at the primordial stage of the leaves. We also think that the marginal meristem of a developing leaf of a barnyardgrass seedling is very easily and, in fact, very highly vulnerable to benthicarb and constitutes the primary site of benthicarb-action.

The swelling of the coleoptile all around its base involved no cell proliferation and was caused due to outward folding of the

Fig. 13. Transection of a treated seedling passing through the leaf. Note one of the marginal wings is deflexed slightly upward (indicated by arrow, $\times 310$ approx.).

Figs. 14 & 15. Transections of treated seedlings passing through the leaves. Note one of the marginal wings is inflexed (indicated by thin arrow) and the other is deflexed (indicated by bold arrow, $\times 310$ approx.).

Fig. 16. Transection of a treated seedling passing through the leaf. Note the two marginal wings are truncate meeting end to end without fusion ($\times 310$ approx.).

Fig. 17. Transection of a treated seedling passing through the leaf. Note one of the overlapped marginal wings are remarkably sinuated (indicated by arrow) and the other only slightly ($\times 310$ approx.).

Fig. 18. Transection of a treated seedling passing through the leaf. Note the two overlapped marginal wings are tightly adpressed to each other. Also note an abnormal radial elongation of the adaxial epidermis (indicated by arrow, $\times 310$ approx.).

Fig. 19. Transection of a 10 days' old seedling passing through the sheath region of a tubular first leaf. Note the cells in the vascular bundles are more or less in tangential plane ($\times 80$ approx.).

coleoptile at that region. We consider that it is one of the nastic responses of barnyardgrass to benthioncarb. Exactly a similar phenomenon was also observed by Dawson⁴⁾ in barnyardgrass on treatment with EPTC, which is also a thiocarbamate herbicide. In this regard another point which deserves consideration is that the diverging angle of the coleoptilar vascular bundle from the main axis was much greater in the treated coleoptile than in the untreated one. Why there occurred such a difference? Was the course of vascular bundle determined by the orientation of the coleoptile or vice versa? These, however, were not well understood and further investigations are required to explore these interesting problems.

The increment of lateral appendages near the shoot apex and at the leaf axils, as observed in this study, substantiates our observations on the morphology of benthioncarb treated barnyardgrass plants in which a remarkable increase in the number of tillers was observed at sub-lethal doses^{12,13)}. The appendages observed in this study are the primary, secondary and, probably also, the tertiary tillers, which perhaps were stimulated to initiate but the further growth of which were inhibited by the higher concentration of the herbicide. Similar stimulation of axillary buds were also observed by other workers in herbicide treated plants^{1,6,7)}. Such phenomenon in a herbicide treated plant is thought to be caused by the loss of apical dominance of the main shoot and the hormonal imbalance in the injured plant.

The undulation of the leaf surface and the sinuation of the leaf are the two different phenomena. The former was induced due to differential elongation and division of cells at the adaxial side of the leaf, whereas the cause of the latter was not well understood. However, in a sinuated leaf, the linear rows of the cells were buckled and the inner and outer walls of individual cell elongated differentially. But it was not clearly understood whether these were the cause of the sinuation of the leaf.

The adnation which was caused by the longitudinal fusion of two adpressed leaves

might have been the result of initiation of two successive leaf primordia approximately at about the same level on the shoot apex. Exactly a similar phenomenon was also observed by Holt⁵⁾ in 2,4-D treated *avena* seedlings.

Our observation in this study also add some new informations on the effect of benthioncarb on barnyardgrass shoot apex. We observed that the herbicide caused the cells in the corpus and tunica to vacuolate extensively and to elongate abnormally resulting an elongation of the shoot apex as a whole and induced periclinal divisions in the cells of tunica on the tip and near the tip without any organization of leaf primordia at the region of these divisions. Similar observations on the shoot apex of a herbicide treated plant have no one yet reported. We, however, observed exactly a similar effect of benthioncarb on a rice shoot apex¹⁴⁾. Though we did not observe necrosis of cells in the shoot apex as observed by Shibayama and Worley¹⁶⁾, we observed extensive vacuolation and enlargement of cells in the shoot apex.

From this study it was thus, revealed that benthioncarb exerted profound morphogenetic effects on barnyardgrass. In the shoot apex the herbicide caused extensive vacuolation and abnormal elongation of cells and induced cell division in abnormal sites and planes. These abnormalities in the growth of cells in the shoot apex might have suppressed the further growth of the shoot apex as a whole, stimulating the growth of lateral bud initials and inducing abnormal growth of marginal initials of leaf primordia with the consequence of increased lateral appendages, formation of tubular leaf, and other growth aberrations in the developing leaf primordia. Almost all of the growth aberrations induced by benthioncarb in barnyardgrass were more or less similar to those induced by hormone herbicides in susceptible plants. We, therefore, conclude that either benthioncarb itself or its degradation products possess hormonal properties or acquire growth regulating activities in the plant body so as to induce several morphogenetic effects on barnyard-

grass.

Summary

The histological effects of benthicarb on barnyardgrass plants were reported and discussed. The salient features are stated below.

1. Benthicarb exerted profound effects on the marginal growth of the developing leaf resulting the induction of tubular leaf, and change of overlapping manner, inflexion, deflexion, truncation, sinuation and tightly overlapping of the marginal wings. We considered the marginal meristem to be the primary site of benthicarb-action.

2. The herbicide induced swelling at the coleoptilar base sinuation of the leaf, undulation of the leaf surface, adnation of two successive leaves, necrosis of the leaf tissues, and distorted the upward growth of the leaf and of the vascular bundle.

3. The herbicide induced periclinal divisions in tunica-cells near the tip and at the tip of the shoot apex. It also caused extensive vacuolation and abnormal elongation of the cells in the shoot apex resulting an elongation of the shoot apex as a whole.

4. Almost all of the histological effects of benthicarb on barnyardgrass were more or less similar to those of hormone herbicides on susceptible plants. We, therefore, concluded that though benthicarb is not a hormonal herbicide either it itself or its degradation products might have possessed hormonal properties or acquired growth regulating activities in the plant body so as to induce morphogenetic effects on the barnyardgrass plants.

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〔和 文 摘 要〕

除草剤ベンチオカーブに関する研究

第4報 ヒエにおよぼすベンチオカーブの組織的影響

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除草剤ベンチオカーブがヒエにおよぼす影響について、組織的な観察を行った。

その結果、ベンチオカーブを処理した個体では、(1) 発育中の葉の周縁生長に異常がおこり、筒状葉や、葉周縁部に抱合様式の変化、内側屈曲、外側屈曲、截形化、波状化や密着などの現象を結果している。(2) 鞘葉基部の隆起、葉の蛇行状弯曲、葉表面の波状起伏、連続する二枚の葉の癒着、葉組織の壊死、葉の垂直伸長や維管束発達の歪曲などがみられる。(3) 茎頂鞘層部の尖端附近、ときには最尖端部に並側分裂が誘発されており、茎頂組織の細胞にいちじるしい液泡化や異常な伸長生長が認められ、茎頂は全体的に長い形となる、などの諸点が明らかになった。

これらの結果は、ホルモン系の除草剤の作用にきわめて類似したものであり、ベンチオカーブは非ホルモン系除草剤であるが、それ自体またはその分解産物が何らかの機作を通して生長調節的な作用を発揮し、ヒエに形態形成的な影響をおよぼすものと考えられる。