Studies on a Herbicide, Benthicarb

II. Effects of different amounts of benthicarb treated at different growth stages on the morphogenesis of barnyardgrass

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Received March 27, 1978

Benthicarb [S-4 (chlorobenzyl) N, N-diethylthiolcarbamate], a selective, non-hormonal herbicide, has growth regulating properties, too. Shigematsu and Kimura³⁵⁾ reported that the herbicide produced tubular leaf by fusion of the wings but it had no effect on the shoot apex of the injured barnyardgrass plants. Shibayama and Worley³⁶⁾ observed necrosis in the shoot apex and induction of tubular-like leaves without fusion of the wings in a benthicarb treated barnyardgrass plant. To re-examine these points of discordance and to make a detailed survey on the growth aberrations, we have investigated the effects of benthicarb on barnyardgrass.

In the preceding paper, we have described the general responses of the barnyardgrass plants to benthicarb. In this paper we shall present the mode of occurrence of those aberrations in barnyardgrass at different growth stages treated by different amounts of the herbicide.

Materials and Methods

The experiments concerning to this paper are the same as those stated in the preceding paper. Before and after the herbicide treatments in the respective growth stages, the plants were very carefully observed for any change in the morphology of the foliar parts. Thus, the sequential events on the occurrence of different aberrations in barnyardgrass plants induced by benthicarb were noted. On the 10th day of the herbicide treatments in the respective growth stages, the number of shoot and leaf per plant was counted and the plant height was

measured. At seed maturity, the plant height and panicle length were measured; the number of shoot and panicle per plant, and the number of primary branches and spikelets per panicle were counted. Data collected on each character was only from 10 individuals that were selected randomly and the mean value is presented.

Results

The mode of occurrence of the aberrations according to the growth stages at which the barnyardgrass plants were treated by different amounts of benthicarb, has been presented below.

1. Pre-emergence stage At pre-emergence, benthicarb suppressed the growth of the seedlings at all doses. At doses higher than 1 kg/ha a.i. although the seeds germinated, the shoot growth was inhibited and no leaf could emerge from the coleoptile. The coleoptile swelled and looked dark green and glossy. At 1 kg/ha a.i. of the herbicide, only the first leaf could emerge and that too only partly; invariably the seedlings died within 15 days of treatment. The herbicide at doses of 0.5, 0.25 and 0.125 kg/ha a.i. induced striking morphological abnormalities. At 0.5 and 0.25 kg/ha a.i. most of the seedlings died within 20 days of treatment and, at 0.125 kg/ha a.i. most of the seedlings recovered from the injury. But the seedling height was reduced, emergence of leaf was delayed and the number of shoot was increased (Table 1).

A remarkable swelling was also observed at the coleoptilar node of the seedlings. Morphologically, the first leaf was tubular

and this hindered the emergence of the second leaf which ultimately kinked; formed loop and distorted. The third leaf also formed loop and kinked. The fourth leaf could emerge and unfold but became shorter and broader, and probably, thicker than that of the untreated one; also it tended to curl. The whole plant looked dark green. Leaves from the fifth one were more or less normal as those of the untreated ones. The first 3 or 4 leaves of the first 3 or 4 primary or secondary tillers also showed abnormalities like tubular structure, kinking, looping and curling. However, at seed maturity, benthocarb at the rate of 0.125 kg/ha a.i. produced shorter plants and panicles, less number of primary branches and spikelets per panicle and more number of tillers than those of the untreated plants.

2. 10-day stage Ten days after sowing when the plants had, on an average, a leaf number of 3, the plants were treated with 0.125 and 1.0 kg/ha a.i. of the herbicide. After 4 days of treatment at the dose of 0.125 kg/ha a.i. the plants lost vigour and faded. The whole of the sheath and a part of the lower portion of the lamina of the 4th leaf emerged being tubular. On the 5th day, the basal portion of the seedling seemed to be tightly enclosed by the tubular 4th leaf and the seedling started to bend downwards at the tubular region of the 4th leaf just above the laminal joint. On the 6th day, the seedlings completed bending and the shoot at the laminal region of the leaves fell over the soil surface; also the 4th leaf at the region of curvature distorted. Through this distorted region, a part of the 5th leaf being longitudinally rolled came out and grew to form a loop. In some individuals, the 6th leaf followed the same phenomenon as that of the 5th one. Within 7 days of treatment, all the treated plants turned dark green and each of the plants started to produce tiller profusely. Tillers came out breaking through the sheath all around the seedling base. All the tillers were dark green and swollen at the base. The first 4 or 5 tiller-leaves were never normal in appearance and were always narrower and shorter than those of the

untreated ones. In some individuals, the first tiller-leaves had sheath-like appearance. In some cases, the 2nd tiller-leaves remained longitudinally adpressed over the first tiller-leaves; sometimes, the margins of these adpressed leaves emerged being longitudinally fused. In some individuals, the lamina-joint of two wings of a tiller-leaf differentiated separately so that the same of one wing developed in a position much lower than that of the other wing. In a tiller, formation of tubular and looped leaves, kinking and curling of leaves were very common phenomena for the first 4 or 5 leaves. All the tiller-leaves in a treated plant looked shorter than those of the untreated ones. However, within 7 days of treatment the 4th leaf, which enclosed the main shoot, started to die-back and within 15 days died completely. In some individuals, the main shoot also died, leaving many tillers around the base. Sometimes, the 5th leaf also died-back while the 6th leaf could recover from the injury. The leaves above the 5th or 6th one in the main shoot could not be identified due to the similarity in appearance with the tiller-leaves. At 1 kg/ha a.i. of the herbicide, there was no difference in the type of aberrations but the phenomena were induced at least one day earlier than, and apparently to a severe extent to, those in 0.125 kg/ha a.i. of the herbicide. However, on the 10th day of treatment, it was observed that the plant height and leaf number were remarkably reduced, and the shoot number was remarkably increased (Table 1). Reduction in plant height and leaf number was more pronounced at 1 kg/ha a.i.. And, at seed maturity, the plant height, panicle length, and number of primary branches and spikelets per panicle were remarkably reduced with a remarkable increase in the number of shoot and panicle per plant (Table 2). No abnormality in floral structure was observed in plants treated at this stage.

3. 20-day and 30-day stages More or less similar responses of the main and axillary shoots but apparently to a less extent to those in the 10-day stage were observed in these stages. Effects of the herbicide in 20-

Table 1. Effect of benthicarb on plant height, and production of shoot and leaf on the 10th day of treatment at different growth stages.

Growth stage	Dose (kg/ha a.i.)	Plant height*	Shoot number	Leaf number
Pre-emergence	Control	12.41	1.20	2.97
	0.125	4.84 (−61.00)**	2.00 (+66.67)	3.00 (+1.01)
10-day stage	Control	51.23	3.50	6.50
	0.125	23.97 (−53.21)	9.90 (+182.86)	4.70 (−27.37)
	1.0	16.56 (−67.68)	9.30 (+165.71)	4.10 (−36.92)
20-day stage	Control	72.66	3.83	8.55
	0.125	62.01 (−14.66)	5.50 (+43.60)	8.00 (−6.43)
	1.0	54.92 (−24.42)	5.80 (+51.44)	7.89 (−7.72)
30-day stage	Control	79.97	5.50	10.48
	2.0	72.08 (−9.85)	6.30 (+14.55)	9.58 (−8.59)
	4.0	71.37 (−10.75)	6.00 (+9.09)	9.17 (−12.50)
40-day stage	Control	116.64	5.20	13.91
	2.0	114.93 (−1.47)	5.50 (+5.77)	13.90 (−0.07)
	4.0	114.12 (−2.16)	5.80 (+11.54)	13.94 (+0.22)

* Plant height in centimeter.

** Figures in the parentheses indicate percent increase (+) or decrease (−) in comparison to the respective value in control plant.

day stage were visible from the 7th leaf and those in the 30-day stage were visible from the 9th leaf, and these two leaves in the respective stage started to die-back from 10 days of treatment and in some individuals, died completely by 20 days of the treatment.

Kinking, looping, tubular structure and curling occurred in first 3 or 4 leaves emerging after the treatment. All the tiller-leaves were shorter than those of the untreated ones and were malformed; tubular, kinked, partially emerged, looped, twisted, curled, narrow and pointed, and longitudinally adpressed over the successive lower leaves. The most note worthy responses in these stages, were the malformations in the

spikelet structures as those stated in the preceding paper. We did not explore the frequency of occurrence of the abnormal spikelets as to the herbicidal doses and plant growth stages but in a limited inquiry we observed that the abnormal spikelets occurred more frequently in plants treated at 20-day stage and in plants treated at lower dose in a particular stage. In a panicle or in a primary branch of the panicle, the abnormal spikelets occurred more frequently towards the tip. However, on the 10th day of treatment, it was observed that the plant height and leaf number were decreased and the number of shoot and panicle was increased in plants treated at both 20-day and 30-day stages but to a less extent to those in 10-day stage (Table 1). In these stages also, similar to those in 10-day stage, the plant height, panicle length, and number of primary branches and spikelets per panicle were remarkably reduced at seed

maturity, with a remarkable increase in the number of shoot and panicle (Table 2).

4. 55-day stage In this stage no appreciable reduction or increment in plant characters was observed at any dose of the herbicide (Tables 1 & 2). However, at 4 kg/ha a.i. the 2nd leaf below the flag leaf started to die-back after 7 days of treatment. The first leaf below the flag leaf turned tubular and this obstructed the normal emergence of the flag leaf and, in turn, of the panicle. Thus, panicle also formed a loop. The primary branches of the panicle in some individuals remained adhere to each other and did not spread as those in the untreated ones. In some individuals, the spikelets in the tiller-panicle could not

Table 2. Effect of benthicarb on plant characters of barnyardgrass at seed maturity.

Growth stage	Dose (kg/ha) a. i.	Plant characters					
		Plant ht.*	Shoot number	Total pp.*	Panicle length	Number of pb.*	Number of sp.*
	Control	117.27	5.30	3.70	17.90	50.60	1,398.60
pre-emergence	0.125	116.11 (-0.99)**	8.40 (+58.49)	5.80 (+56.76)	13.48 (-24.69)	28.00 (-44.66)	681.20 (-51.29)
10-day stage	0.125	83.64 (-28.68)	24.40 (+360.38)	19.56 (+428.65)	9.00 (-44.69)	22.00 (-56.52)	360.00 (-74.26)
	1.0	73.74 (-37.12)	21.80 (+311.32)	17.00 (+359.46)	9.33 (-47.88)	15.17 (-70.02)	204.00 (-85.41)
20-day stage	0.125	58.15 (-50.41)	17.20 (+214.53)	14.00 (+278.38)	11.00 (-38.55)	19.00 (-62.45)	397.40 (-71.59)
	1.0	56.40 (-51.91)	19.70 (+271.70)	15.60 (+221.62)	9.82 (-45.14)	14.00 (-72.33)	215.00 (-84.61)
30-day stage	2.0	56.58 (-51.75)	19.20 (+262.26)	15.80 (+327.03)	10.24 (-42.79)	19.60 (-61.26)	209.20 (-85.04)
	4.0	52.13 (-55.55)	18.20 (+243.40)	15.81 (+310.81)	10.58 (-40.89)	12.60 (-75.10)	117.20 (-91.62)
55-day stage	2.0	115.60 (-1.42)	5.70 (+7.55)	3.90 (+5.41)	17.22 (-3.80)	46.60 (-7.91)	1156.60 (-17.30)
	4.0	114.32 (-2.51)	5.88 (+10.94)	4.11 (+11.08)	17.58 (-1.79)	44.80 (-11.66)	1174.60 (-16.02)

* ht=height in centimeter, pp.=panicle per plant

pb.=primary branch per panicle, sp.=spikelet per panicle

** Figures in the parentheses indicate percent increase (+) or decrease (-) in comparison to the respective value in control plant.

fertilize and the panicle as a whole turned white; this happened due to complete death of the shoot. In most of the individuals, tillers grew abnormally around the base of the plant after heading in the main shoot. All these abnormalities in 2 kg/ha a.i. were confined to the tillers only and occurred to a very small extent.

Discussion

Benthicarb, a thiocarbamate herbicide, unlike other thiocarbamates, it can be very effectively used in rice field^{2,6,7,14,20,21,22,30,38,43}. But the effects of this herbicide on barnyardgrass are more or less similar to those of the other thiocarbamates on susceptible plants^{3,5,19,29}. Dowson⁵ observed that EPTC, a thiocarbamate herbicide, induced kinking of mesocotyl in barnyardgrass and concluded that the kinking was caused by a decreased ability of the

injured coleoptile to penetrate the soil. The kinking of leaves in a benthicarb treated barnyardgrass seedling as observed by us may be partly due to inability of the younger, inner leaves to emerge through the older tubular leaves that remained adpress on the younger leaves and partly due to differential growth of the leaf tissues.

As to the structure of tubular leaf in a benthicarb treated plant we agree to Shigematsu and Kimura³⁵ and disagree to Shibayama and Worely³⁶. Formation of tubular leaf due to herbicidal treatment is not uncommon. Auxin herbicides cause fusion of the leaf margins at primordial stage and eventually, cause the formation of tubular leaf^{10,15,16}. Other instances of herbicide induced tubular leaves are due to partial unfolding³⁹ or complete rolling of leaves^{1,17,18}. To determine how and when a tubular leaf was formed in a benthicarb

treated plant we observed the plants very carefully. Any leaf or any part of the leaf, which was already unfolded at the time of treatment never induced to form a tubular structure. The wings of the leaves, which were elongating through the older leaves at the time of treatment, were induced to inflex and overlap or fuse and thus, emerged as tubular structure. We consider that the induction takes place during the marginal growth of the wings at the primordial stage of the leaf and that the fusion of wings results from the abnormal radial elongation of cells of the overlapped wings.

Induced sprouting of axillary buds due to chemical treatments has also been reported by many workers^{4,12,13,26,37,40}.

Such phenomenon in a chemically treated plant may be attributed to hormonal imbalance in the plant body or loss of apical dominance due to damage in the main shoot. We, therefore, consider that if benthicarb to be applied in a crop field to control barnyardgrass at post emergence stages, the treatment should be done at sufficiently lethal dose otherwise, the crop may face a severe competition with tillers, which will grow in a slightly injured plant, for growth requirements.

Induction of abnormalities in the spikelet structures may indicate that the herbicide can influence the morphogenesis in the reproductive shoot apex similar to those of other growth hormones^{8,9,11,23,24,25,27,28,31,33,34,41,42}. Because, that the vegetative shoot apex in barnyardgrass is transformed to reproductive shoot apex on about 30 days after sowing, that the aberrations in spikelet structures occurred in plants treated at 20-day and 30-day stages only, and that the abnormalities occurred in tips of both main axis and primary branches of the panicle, it may be thought that the herbicide can affect the morphogenesis of floral shoot apex, only in the early stage of morphogenesis in the reproductive shoot apex.

We, therefore, consider that at sub-lethal doses, the herbicide disrupts or modifies the growth processes in barnyardgrass as the auxin herbicides do in susceptible plants and at lethal doses, the severity of the effects

reaches to such an extent that the plants die. These growth regulating effects of benthicarb are of great significance to the students of plant morphogenesis and need a thorough investigation in relation to different aspects of growth.

Summary

The mode of occurrence of the aberrations at different growth stages of barnyardgrass plants treated with different amounts of benthicarb were studied. The results are summarized below.

1. Benthicarb, a non-hormonal herbicide, produced morphogenetic effects on barnyardgrass at all growth stages and at all doses.

2. The aberrations produced differed according to growth stages at which the plants were treated. The malformations in the higher doses were produced earlier than, and to severe extent to, those in the lower doses.

3. In pre-emergence, at lethal doses, the herbicide did not inhibit the germination of the seeds but inhibited the development of the young seedlings by preventing the leaves from emerging from the coleoptile. At sub-lethal doses, the herbicide retarded the growth of the seedlings and produced several aberrations in the leaves; bending, curling, formation of tubular structure, distortion of leaves, formation of loop, kinking, and shortening, thickening and broadening of the leaves.

4. At post-emergence stages, besides the above stated malformations, the herbicide produced adnation of two leaves or longitudinal fusion of margins of two leaves, separation of laminal joints of two wings in a single leaf, shorter and narrower leaf, and shorter panicles with malformed spikelets.

5. Abnormality of any kind was never observed in any leaf unfolded before the herbicide treatment. Only the leaves, which were emerging at the time of treatment or initiated after the treatment showed deformities.

6. Tubular leaf was produced due to inflexion and overlapping or fusion of sheath- and/or lamina-wings continuously or

discontinuously. The induction was thought to occur at primordial stage of the leaf.

7. The particular leaves forming tubular structure differed according to growth stages at which the plants were treated; at pre-emergence- the 1st, 2nd and 3rd leaves, at 10-day stage- the 4th, 5th and 6th leaves, at 20-day stage- the 7th, 8th and 9th leaves, at 30-day stage- the 9th, 10th and 11th leaves and, at 55-day stage- the flag leaf and the two successive lower leaves.

8. Abnormalities in spikelet structures were observed in plants treated at 20-day and 30-day stages only. The abnormal spikelets occurred towards the tips of both the main axis and primary branches of the panicle.

9. Irrespective of growth stages, the herbicide produced shorter plants and panicles, less number of primary branch and spikelet per panicle, and more number of shoots and panicles per plant.

Acknowledgements

We are thankful to Dr. Yoji Takeoka, Assistant Professor, Laboratory of Theory of Agronomy and Plant Breeding, Faculty of Agriculture, Nagoya University, Japan, for his kind help and suggestions during the study. We also thank Dr. Yuji Kawamura, Chief Plant Physiologist, Nissan Chemical Industries, Shiraoka, Japan, for his kind supply of seeds of barnyardgrass. Kumiai Chemical Industry, Shizuoka, Japan, contributed the benthocarb used in this study.

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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2報 処理時期ならびに処理濃度の相違がヒエの
形態形成におよぼす影響A. A. マムン・清水 正 治
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ベンチオカーブの処理濃度や処理時期をかえて、ヒエにあらわれる外部形態的異常の発生状況を調べた。得られた結果は次の通りである。

1. ベンチオカーブは非ホルモン系除草剤であるが、いずれの場合もヒエに対して形態形成的な異常を誘発した。
2. 異常発生は処理時期によって異なり、本実験の範囲では高濃度ほど早く、かつ顕著であった。
3. 出芽前処理においては、致死濃度でも発芽はするが、鞘葉からの葉の出葉が妨げられその後の生育が阻止された。致死濃度以下では、生育が抑制され、葉に弯曲、波状化、筒状化、亀裂、輪状化、よじれなどの異常が現われると同時に、長さが短かく巾の広い厚い葉が形成された。
4. 出芽後処理においては、上記のほか、二つの葉の密着や癒着、一枚の葉のラミナ・ジョイントの分離、巾の細い短葉などがみられるとともに異常小穂をもった短い穂が現われた。
5. 処理前に展開している葉にはいかなる異常も現われず、処理時に伸長期にあるもの、または処理直後発生した葉に異常が現われた。
6. 筒状葉は葉鞘と葉身の両翼、または葉鞘が葉身の両翼が内側にまきこみ、連続的または非連続的に重なりあうか癒着することによって形成される。この筒状葉の形成は葉の始原体期におこると考えられる。
7. 筒状葉の発生する葉位は処理時期によって異なり、出芽前処理では第1, 2, 3葉に、播種後10日期処理では第4, 5, 6葉に、20日期処理では第7, 8, 9葉に、30日期処理では第9, 10, 11葉に、また55日期処理では止葉およびそれ以下第1, 第2葉に現われる。
8. 異常構造をもつ小穂は播種後20日, 30日期処理区のみに見られた。異常小穂は穂の主軸および一次枝梗の尖端部分に多く現われる。
9. 処理時期のいかんを問わず、処理により草丈、穂長の短縮、一穂あたり一次枝梗数、小穂数の減少がみられ、茎数、穂数は増加を示す。