THE INFLUENCE OF PLANT GROWTH REGULATORS APPLIED TO BARLEY DURING MALTING ON PROPERTIES OF MALT[1]

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ABSTRACT

The following materials were applied to steeped barley immediately before malting: gibberellic acid (GA), chlorocholine chloride (CCC), coumarin (Cn), hexachlorophene (Hcp), 2,3-benzoxazolinone (Bao), 3-amino-1,2,4,-triazole (AT), polymyxin (Pn), kinetin (Kn), and 3-indolyl acetic acid (IAA). All of the malts and the beers produced from selected ones were examined for the usual quality characteristics.

The most pronounced changes were caused by GA, CCC, AT, and Kn. GA stimulated growth generally; CCC inhibited it. AT stimulated all manifestations of growth except rootlet and acrospire growth. Kn improved some desirable properties but decreased beta-amylase activity and increased acrospire growth. IAA increased only laboratory wort nitrogen, alpha-amylase activity, and rootlet growth. Cn and Hcp decreased diastatic power and beta-amylase but increased alpha-amylase; their effect on rootlet growth depended upon the concentration applied. Worts and beers from malts treated with GA, Kn, and AT were slightly dark.

The results suggest that there may be situations in which some of these or similar materials could be used in malting.

During the last 35 years many substances, some of which occur naturally in plants, have been shown to affect markedly the growth behavior of plants when present in very small amounts (3). However, in previous experiments (10) the treatment of barley during malting with low levels of some of these compounds (3-indolyl acetic acid, naphthaleneacetic acid and several of its esters, and naphthaleneacetamide) did not alter appreciably the properties of the finished malt except at high levels of application for which a general inhibition of growth occurred. In the present work, 3-indolyl acetic acid and eight additional growth regulators were examined for their influence on the properties of malt and beer when applied to barley at the beginning of the malting process.

Materials and Methods

The growth regulators used and their sources[2] were as follows:

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[2]Mention in this publication of a trade product, equipment, or a commercial company does not imply its endorsement by the U. S. Department of Agriculture over similar products or companies not named.

gibberellic acid (GA), Eli Lilly and Co., Indianapolis; 2-chloroethyltrimethyl ammonium chloride (chlorocholine chloride) (CCC), American Cyanamid Co., New York; coumarin (Cn), Eastman Organic Chemicals, Rochester, New York; bis-(3,5,6-trichloro-2-hydroxyphenyl) methane (hexachlorophene) (Hcp), Sindar Corp., New York; 2,3-benzoxazolinone (Bao), Eastman Organic Chemicals, Rochester, New York; 3-amino-1,2,4-triazole (AT), California Corp. for Biochemical Research, Los Angeles, California; polymyxin B sulfate (Pn), a mixture of cyclic polypeptides produced by *Bacillus polymyxa* (Prazmowski) Migula, Chas. Pfizer & Co., Inc., New York; 6-furfurylaminopurine (kinetin) (Kn), California Corp. for Biochemical Research, Los Angeles, California; 3-indolyl acetic acid (IAA), Nutritional Biochemicals, Cleveland, Ohio.

Traill barley of the 1959 crop was malted in quantities of 225 g., dry basis, by the micromalting procedures of Shands, Dickson, and Dickson (28). After steeping for 24 hours the barley was drained and the plant growth regulator, in 10 ml. of solvent, was sprayed evenly on the barley to bring the moisture level to approximately 45%. A control for each applied material was prepared similarly with the appropriate solvent. The solvents used were 50% aqueous ethanol for Cn and Hcp, 80% aqueous ethanol for Bao, 33% aqueous ethanol for IAA, 0.006*N* hydrochloric acid for Kn, and water for GA, CCC, AT, and Pn. Each plant-growth regulator was applied at five concentration levels in addition to the control. Replications were seven for the control and five for each concentration of growth regulator. The capacities of the malting chamber and kiln were such that 32 malts (seven for controls and five for each of the five concentrations of one growth regulator) could be accommodated in one experiment. The following characteristics of malt were examined in the usual way (2): extract, wort color, wort nitrogen, diastatic power, and alpha-amylase. The method of Olson *et al.* (25) was used to measure beta-amylase. Growth index, loss due to steep and respiration, loss due to rootlet growth, and malt recovery were determined by the methods of Shands *et al.* (29). The analytical results were examined for significant differences by variance analysis.

For each growth regulator, beer was prepared by using the composited control malts and the composited malts which had received the growth regulator at the highest concentration. In addition, for the experiment with GA, beer was made from malt that had received a treatment at an intermediate concentration. The microbrewing technique of Burkhart, Otis, and Dickson (6) was used. Treated malt and the appropriate control were microbrewed on the same day. The fol-

lowing characteristics of brewery wort and beer were examined by established methods (2,6): extract, color, pH, formol nitrogen, total nitrogen, maltose, and dextrin. Beers also were analyzed for alcohol, degree of fermentation, clarity stability, and gas stability. Nitrogen values were adjusted, where applicable, for nitrogen added as growth regulator nitrogen.

To estimate experimental error for each brewing analysis, the standard deviation for the nine controls was calculated. For each brewing analysis an arbitrary level of significance was established by considering the mean value for malt treated with a growth regulator to be significantly different from the corresponding control mean if the mean for the treated malt differed from the corresponding control mean by at least three times the standard deviation for the nine controls.

Results and Discussion

The means and least significant differences as determined by variance analysis for malt characteristics affected by treatment are given in Table I.

The microbrewing procedure is a complex microbiological assay involving manipulations in which small variations may cause unpredictable changes in the properties of wort and beer; so interpretation is difficult in comparing results from a single microbrew of a treated malt with a single microbrew of the control malt. Yet the nature of the process is such that adequate replication could not be attained except for the control malts.

For brewing data the establishment of the arbitrary level of significance as previously described allows at least some attention to be directed toward the changes of greater magnitude. The nine controls were brewed on different days; so some variation in yeast inoculum may have occurred. Furthermore, the liquids applied to these control malts after steeping differed in some cases, depending upon the solvent used for the growth regulator applied in the corresponding treatment. These sources of variation among controls did not exist between a treatment and its control. Hence if a treatment caused a change, in comparison with the corresponding control, that exceeded three times the standard deviation for the control data as a whole, the treatment can be considered effective, as least tentatively. Those analyses of brewery wort and beer that were significantly different on this basis are presented in Table II.

A qualitative summary of all malting and brewing analyses influenced by treatment appears in Table III.

TABLE I

MEANS AND SIGNIFICANT DIFFERENCES FOR MALT ANALYTICAL FACTORS SIGNIFICANTLY INFLUENCED BY GROWTH REGULATORS[a]

REGULATOR AND CONCENTRATION	STEEP AND RESP. LOSS	ROOTLET LOSS	MALT RECOVERY	GROWTH INDEX	EXTRACT	WORT N	DIASTATIC POWER	BETA-AMYLASE	ALPHA-AMYLASE	WORT COLOR
	%	%	%		%	%	°L	*Maltose Equiv.*	*20 ° Dext. units*	*° Lov. 52*
GA										
0	4.7			93	76.2	0.62	162	518	39	1.4
3.25×10^{-5} (0.050)								541		1.6
6.5×10^{-5} (0.10)	5.2			90			173	545	44	1.6
3.25×10^{-4} (0.50)	5.6			95	77.9	0.78	182	549	56	1.7
6.5×10^{-4} (1.0)	5.4			95	78.2	0.80	184	540	61	1.7
3.25×10^{-3} (5.0)	5.4			97	78.8	0.90	194		76	1.9
LSD	0.5			2	1.3	0.13	7	18	2	0.2
CCC										
0					76.3	0.65	169	539	41	
3.9×10^{-5} (0.027)										
3.9×10^{-4} (0.27)									38	
3.9×10^{-3} (2.7)						0.62			37	
1.43×10^{-2} (10.0)					75.9	0.61	154	507	34	
2.86×10^{-2} (20.0)					75.5	0.59	153	503	34	
LSD					0.4	0.03	6	23	2	
Cn										
0	5.1	3.2	91.8	94		0.66	162	524	37	
3.94×10^{-5} (0.0256)		3.1	92.0	89						
3.94×10^{-4} (0.256)		3.1		90		0.67	159			
3.94×10^{-3} (2.56)	5.4	3.1				0.67	159			
1.43×10^{-2} (9.29)	5.4	3.1	91.6			0.67	158	502	39	
2.86×10^{-2} (18.58)	5.4	2.9		91		0.68	157	488	41	
LSD	0.2	0.1*	0.2*	3		0.01	3*	20	2	
Hcp										
0	4.6	3.0	92.5		76.0		164	528	38	
3.94×10^{-5} (0.071)										
3.94×10^{-4} (0.71)	4.8	2.9			76.3					
3.94×10^{-3} (7.1)	4.8	3.2	92.2		76.6			502	42	
1.42×10^{-2} (25.7)	4.9	3.1	92.2		76.6		160		40	
2.86×10^{-2} (51.8)	4.8		92.3				155	488	40	
LSD	0.1	0.1	0.2		0.3		4	23	2	

(continued)

TABLE I (*Continued*)

Regulator and Concentration	Steep and Resp. Loss	Rootlet Loss	Malt Recovery	Growth Index	Extract	Wort N	Diastatic Power	Beta-Amylase	Alpha-Amylase	Wort Color
	%	%	%		%	%	°*L*	*Maltose Equiv.*	*20 ° Dext. units*	°*Lov. 52*
Bao										
0	4.6	3.1	92.4	86	76.6	0.70				
2.86×10^{-2} (17.18)	4.7									
1.43×10^{-1} (85.9)	4.7									
2.86×10^{-1} (171.8)	4.8									
5.72×10^{-1} (343.6)	4.8				76.9	0.72				
1.14 (684.8)		2.2	93.3	81	76.8					
LSD	0.1	0.5	0.5	3	0.2	0.02				
AT										
0	5.3	3.4	92	91	76.4	0.67	163	511	42	1.4
3.94×10^{-3} (1.47)			91						46	
1.43×10^{-2} (5.35)			93		77.6	0.82	171		55	
2.86×10^{-2} (10.7)		1.7	93		78.1	0.89	174		52	1.8
5.74×10^{-2} (21.5)		1.4	94	81	77.8	0.88	172	550	46	1.9
1.14×10^{-1} (42.7)	4.9	1.2	94	75	77.2	0.86				1.8
LSD	0.2	1.5	1	8	0.8	0.13	6	19	2	0.3
Pn										
0							159	498		
0.157 (0.698)										
1.57 (6.98)							168	528		
6.0 (26.7)										
12.1 (53.8)							164	514		
24.2 (107.6)										
LSD							5*	16*		
Kn										
0	5.1	3.5	91.6	89	76.2	0.66		511	43	1.4
3.94×10^{-4} (0.38)	4.9		91.8							
3.94×10^{-3} (3.8)	5.0		91.8							
1.43×10^{-2} (13.7)	5.0		91.8	94		0.70				
2.86×10^{-2} (27.4)		3.3	91.8	92		0.70			47	
5.72×10^{-2} (54.8)		3.0	92.0	94	76.6	0.73		492	51	1.6
LSD	0.1*	0.2	0.2	3	0.3	0.04		13*	2	0.1
IAA										
0		3.4	92.1			0.661			40.2	
1.23×10^{-4} (0.096)										
1.28×10^{-3} (1.0)		3.5								
3.85×10^{-3} (3.0)		3.6	91.7			0.671				
6.41×10^{-3} (5.0)		3.6	91.7						41.6	
1.28×10^{-2} (9.98)		3.6	91.6						41.7	
LSD		0.1*	0.3			0.008			0.9*	

[a] Concentrations are mmol. per 225 g. barley d.b. except for Pn, for which the concentration is expressed as mg. per 225 g. barley d.b. Values in parentheses are the concentrations expressed as p.p.m. Asterisk denotes least significant difference (LSD) at 5% level; other LSD at 1% level; absence of data denotes lack of significance.

TABLE II

BREWERY WORT AND BEER CHARACTERISTICS SIGNIFICANTLY AFFECTED BY GROWTH REGULATORS[a]

REGULATOR AND CONCENTRATION	WORT			BEER		
	Color	Total N	Formol N	Color	Total N	Formol N
	°*Lov.* 52	% *w/w*	% *w/w*	°*Lov.* 52	% *w/w*	% *w/w*
GA						
0 (GA control)	2.0	0.071	0.018	1.8	0.049	0.006
6.5×10^{-5} (0.10)	2.4			2.1	0.057	
3.25×10^{-3} (5.0)	3.1	0.103	0.029		0.076	0.015
Bao						
0 (Bao control)					0.052	0.008
1.14 (684.8)					0.062	0.011
AT						
0 (AT control)	2.0	0.076	0.021	1.9	0.054	0.009
0.114 (42.7)	3.3	0.099	0.029	3.1	0.075	0.016
Kn						
0 (Kn control)	2.2	0.071	0.019	1.8	0.051	
5.72×10^{-2} (54.8)	2.5	0.084	0.022	2.2	0.061	
Control mean	2.1	0.075	0.019	1.7	0.051	0.007
LSD[b]	0.3	0.008	0.003	0.3	0.006	0.003

[a] Concentrations are mmol. per 225 g. barley d.b. Values in parentheses are the concentrations expressed as p.p.m.
[b] For significance see text.

TABLE III

SUMMARY OF SIGNIFICANT EFFECTS OF GROWTH REGULATORS ON MALTING CHARACTERISTICS AND ON MALT, WORT, AND BEER COMPOSITION[a]

GROWTH REGULATOR	MALTING CHARACTERISTICS				MALT ANALYSES					
	Steep and Resp. Loss	Rootlet Growth	Malt Recovery	Growth Index	Lab. Wort Color	Lab. Wort Extract	Total N of Lab. Wort	Diastatic Power	Beta-Amylase	Alpha-Amylase
GA	+			+−	+	+	+	+	+	+
CCC						−	−	−	−	−
Cn	+	−	+−	−			+	−	−	+
Hcp	+	+−	−			+		−	−	+
Bao	+	−	+	−		+	+			
AT	−	−	+	−	+	+	+	+	+	+
Kn	−	−	+	+	+	+	+		−	+
Pn								+	+	
IAA		+	−				+			+

	BREWERY WORT ANALYSES			BEER ANALYSES		
	Color	Total N	Formol N	Color	Total N	Formol N
GA	+	+	+	+	+	+
Bao					+	+
AT	+	+	+	+	+	+
Kn		+	+	+	+	

[a] + and − denote an increase and decrease respectively in comparison with the appropriate control value. +− denotes both changes depending upon the concentration of growth regulator used.

The results obtained with each growth regulator will be discussed separately.

Gibberellic Acid (GA). The practical application of this compound in malting has been explored during the last five years (9,11). GA or its salt acts as a stimulator of growth in general. Enzyme activities are enhanced, particularly alpha-amylase activity, making the application useful in the preparation of malt for the distiller. Recently GA or its potassium salt has been accepted as a permissible food additive in brewer's malt. The use of these compounds has been considered for the malting of barleys which otherwise would produce malt of unsatisfactory enzyme activity (11).

The manner in which GA and other gibberellins function at the molecular level is not understood. There is some evidence that GA, as it occurs in germinating barley, exists in a bound form (9). The presence of the barley embryo is not necessary for some of the stimulatory effects of GA, since barley endosperm responds to GA in a manner that suggests a hormonal reaction which stimulates the formation of amylase, protease, and cytase (26,27). The site of stimulation appears to be the aleurone cells (22).

The results of the present work are in general agreement with those of other workers. There appears to have been a general stimulation of growth that is reflected in steep and respiration loss, in growth index, and in increased amylase and protease activities. No increase in rootlet growth was observed, a result that agrees with the data of Bawden, Dahlstrom, and Sfat (4) but disagrees with work reported by Dickson (11). Increased color of worts and beers was observed as reported by other workers. Clarity and gas stability of beer were not altered by GA treatment.

2-Chloroethyl-Trimethyl Ammonium Chloride (Chlorocholine Chloride) (CCC). CCC and some of its analogues affect plant growth in a manner opposite to that of the gibberellins (3). The most characteristic growth change is the development of stockier plants with short, thick stems. Because of the similarity in structure of this compound with choline and betaine, there is speculation that it may affect transmethylation (3). However, similar changes in growth behavior have been caused by other quaternary ammonium compounds whose structures do not resemble choline or betaine. There have been no reports concerned with the influence of CCC on the properties of malt and beer.

The results obtained indicate that CCC acts as a general inhibitor of physiological systems associated with protease and amylase activities and perhaps with other enzymatic activity contributing to extract.

At the concentrations used, no change was noted in the physical manifestations of growth.

Coumarin (Cn). Cn is widely distributed in plants. It can act as a stimulator or inhibitor of growth, depending upon the concentration, the plant involved, the stage of growth, and the presence of other compounds which can interact with it. Its mode of action is not known, but the activity of many enzymes is affected by it. Possibly this effect may be the result of inhibition of proteolytic cleavage of enzymogens (3). Uncoupling of oxidative phosphorylation and inhibition of mitosis have been attributed to Cn.

Cn has been reported to retard rootlet growth without affecting other properties of malt (20). As shown in Table I, application of Cn at the higher concentration levels resulted in increased steep and respiration loss, laboratory wort nitrogen, and alpha-amylase. Decreases occurred in growth index, diastatic power, and beta-amylase, again mainly at the higher concentration levels.

Bis-(3,5,6-Trichloro-2-Hydroxyphenyl) Methane (Hexachlorophene) (Hcp). Hcp has been a popular germicide for soaps and cosmetics for several years. Since this compound has been reported to inhibit rootlet formation of cucumber and barley seed (24), it was examined for possible effects upon malt and beer. At some concentrations changes occurred, but they were not large. There were increases in steep and respiration loss, extract, and alpha-amylase, and decreases in recovery of malt from barley, diastatic power, and beta-amylase. No consistent change in rootlet growth occurred. A small decrease was recorded for an intermediate concentration level, but higher levels seemed to stimulate rootlet growth slightly.

Microorganisms present during malting may cause desirable changes in some cases and deleterious ones in others (31). Hcp may exert its effect indirectly through altering microfloral types and population.

Polymyxin B Sulfate (Pn). Pn, a mixture of cyclic polypeptides produced by *Bacillus polymyxa* (Prazmowski) Migula, is an antibiotic effective against gram-negative bacteria. Since some bacteria of this type are known to flourish during malting (30), investigation of this material seemed warranted. Furthermore, Pn has been reported to inhibit rootlet growth and to cause leaching of rootlet constituents (23).

At the concentrations used in the present study, Pn caused only small increases in diastatic power and beta-amylase, both at intermediate levels of Pn.

2,3-Benzoxazolinone (Bao). Bao and the 6-methoxy analogue were

thought to be in plant tissues (5,33,35,37) where they afford protection from fungal and insect infestation. Recent work indicates that these compounds are artifacts and that their precursors, 2,4-dihydroxybenzoxazin-3-one and 6-methoxy-2,4-dihydroxybenzoxazin-3-one and the glucosides of these compounds, are the substances in the plant tissue (14,16,17,34,36). However, since Bao and 6-methoxy Bao inhibit the growth of *Fusarium* (5,37), a fungus which can alter malt properties (31), they may affect malt and beer characteristics indirectly by influencing the malt microflora.

The application of Bao to barley inhibited rootlet growth and respiration loss, and increased malt yield and extract (21). Rather large decreases occurred in rootlet growth, but the lack of a statistical evaluation made the remaining changes difficult to interpret. Bao and GA in combination hastened modification and restricted loss due to rootlet growth and to steep and respiration.

The present work tends to support some of these results for the application of Bao alone. Steep and respiration loss increased slightly with increasing Bao concentration. Rootlet loss decreased markedly, which caused an increase in malt recovery for the highest Bao level. Growth index decreased at this Bao level. Extract was increased slightly but only at the high levels of application. There was a very small increase in laboratory wort nitrogen but this occurred at only one of the higher Bao levels. Total nitrogen and formol nitrogen of beer were increased slightly, but these increases were not evident in brewery wort.

3-Amino-1,2,4-Triazole (AT). AT is a phytotoxin used as a defoliant in weed control. The mode of action of this compound is not known, but a number of enzymes are affected by it. Phosphorylase activity with glucose-1-phosphate to form polyglucose was inhibited by the formation of a glucose derivative of AT (12,13). Inhibition of tyrosinase, fatty acid oxidase, lactoperoxidase (8), and catalase (1,18) occurred.

AT has been reported to be both carcinogenic (1,19) and anticarcinogenic (18). In plants there appears to be a resistant reaction that results in a detoxification product (7,15). A study of phosphorus metabolism in barley plants suggested that AT did not affect the phosphate associated with oxidative phosphorylation or of glycolysis but did affect the incorporation of phosphate during the synthesis of nucleic acids (38).

This compound caused a very impressive array of effects upon the properties of malt and beer. In general, enhancement of the desirable properties and diminution of the undesirable ones occurred. Decreases

were found in steep and respiration loss, rootlet growth, and acrospire growth. Increases occurred in recovery of malt, extract, laboratory wort nitrogen, diastatic power, beta-amylase, alpha-amylase, and in total and formol nitrogen of brewery wort and beer. The only changes that may be undesirable were increases in color of laboratory wort, brewery wort, and beer.

Despite the possible carcinogenic properties of AT, the potential of this compound as a growth regulator, rather than a phytotoxin or defoliant, should not be ignored. For example, its use in the production of distiller's malt may be feasible.

6-Furfurylamino Purine (Kinetin) (Kn). Essentially nothing is known about the biochemistry of Kn at the molecular level. A wide variety of growth effects caused by this compound and its analogues have been reported (32). Examples are stimulation of cell division and cell elongation, inhibition of root growth, and stimulation of seed germination. There have been no reports of its influence on barley germinating under malting conditions.

The application of Kn in the present investigation resulted in an interesting array of changes, most of which were small and occurred only with the highest concentration. Growth index increased but rootlet growth and, in some cases, loss due to steep and respiration decreased, which caused an increase in malt recovery. Alpha-amylase was increased, but beta-amylase was decreased. Laboratory wort, brewery wort, and beer had intensified color and an increased level of total nitrogen. Formol nitrogen of brewery wort but not of beer increased. Extract of laboratory wort increased slightly.

3-Indolyl Acetic Acid (IAA). In a previous investigation, IAA at a concentration of up to 10 p.p.m. in the liquid used for steeping and 100 p.p.m. in the liquid used for spraying during malting did not alter the properties of malt (10). However, the quantity of IAA that was retained by the barley was not known. Therefore IAA was included with the growth regulators in the present investigation and applied over a concentration range such that 0.1 to 10.0 p.p.m. based on dry weight of barley (1.23×10^{-4} to 1.28×10^{-2} mmol. per 225 g. of barley) were added to the barley after steep. It is apparent that this concentration range was too limited. At the higher levels of application, rootlet growth increased which resulted in decreased recovery of malt. Alpha-amylase increased at these levels, while laboratory wort nitrogen showed only a small increase at an intermediate level.

Possibilities for Growth Regulators in Malting. In the present experiment, facilities did not permit the application of growth regu-

lators in combinations. There are many reports of antagonistic and synergistic responses in plant growth to the application of two or more growth regulators. The results of the present study and the possibilities of application of growth regulators in combination suggest that situations may arise for which the use of these compounds in malting may be practically and economically feasible.

At present, commercial malting relies upon the availability of high-quality barley capable of producing adequate enzymatic activities. Barley of this quality, however, can be grown only in limited geographical areas. In view of predictions for population increases, it is conceivable that the supply of malting barley may not always meet the demand. In such an economic situation the use of growth regulators may permit the satisfactory malting of high-yielding feed barleys or of barley which, because of an unsuitable growing season, shows poor germination characteristics.

While these and other uses for growth regulators may develop, substantial advances in technology will be limited until there is an understanding of the functions performed by these materials at the molecular level.

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