MALEIC HYDRAZIDE-AUXIN INTERRACTIONS IN WATER UPTAKE OF POTATO DISCS ^{1, 2} RAY TAYLORSON ³ & L. G. HOLM

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Since the introduction of maleic hydrazide (MH) as a plant growth regulator by Schoene and Hoffman (17) there has been some speculation as to its role as an auxin antagonist. Many of the responses to MH of intact or isolated segments of plant tissue suggest some relationship to auxin processes. Thus, the cessation of growth, the prolonging of dormancy and the breaking of correlative inhibition have been related in some way to the auxin process and are also affected by MH. Cell elongation is to be considered similarly. Leopold and Klein (14) were among those who showed that MH had inhibitory effects on this phenomenon as measured in several common auxin bioassays. These workers, have indicated that MH is an antagonist of auxin action. Support for this aspect of MH action comes from the work of Kulescha (13) and Gautheret (8). However, the experimental approach used in these studies was one in which antagonistic effects could readily occur while masking other significant actions.

It is now generally accepted that a significant amount of the activity of auxins on the elongation of plant cells occurs through its influence on the cell wall. A loosening of cell wall constituents followed by an osmotic uptake of water is envisaged with a subsequent elongation of the cell (18). Increased aerobic metabolic activity has been shown to occur also during this period and a variety of cellular reactions have been followed in detail during these various stages under the influence of auxins.

In so far as the results of the present paper are concerned, the initial phases of auxin action are most significant. Other workers have shown that Avena coleoptiles incubated in the presence of auxin and isotonic or slightly hypertonic solutions of mannitol, can display auxin-induced elongation after the sections have been transferred to solutions where elongation may proceed and which contain no auxin themselves (4). These responses have been referred to as residual effects of auxin on cell walls. Such responses may also be readily observed by pretreating potato discs with auxins and following their subsequent uptake of water. This phenomenon has been utilized in the present studies in an effort to determine what effect MH has on this basic process under conditions of pretreatment and continuous incubation.

METHODS & MATERIALS

A technique similar to that used by Hackett and Thimann (11) was used throughout the course of this study. Katahdin potatoes were stored at 7° C until used. Discs 1 cm in diameter and 1 mm thick were cut using a cork borer and a hand microtome. After cutting, the discs were rinsed for 20 minutes in running tap water, given a brief distilled water rinse, and finally placed in petri dishes with enough distilled water to thoroughly wet the surface of the discs but not submerge them, and allowed to stand overnight at 25° C in a dark chamber. After 20 hours, the discs were removed in groups of ten, blotted by a standard procedure of placing them between double thicknesses of paper towel and applying a 100 g weight for 10 seconds, weighed on an electric balance to the nearest ten milligrams, and placed in the apparatus containing the test solution. This marked the beginning of the experimental period. The apparatus consisted of ordinary pyrex petri dishes containing two glass rods 5 mm in diameter, which supported circular pieces of coarse aluminum mesh heavily coated with paraffin. These served as supports for the potato discs. The test solution was added to the dishes until the liquid began to float the paraffin coated screens. This assured identical contact of the discs from dish to dish and thus assured uniform aeration. Each dish containing ten discs comprised an experimental replicate. Three replicates of each test solution were used in all experiments. Critical experiments which were repeated indicated that although the magnitude of the response of the discs varied slightly, the trend of the results was always the same. Data on the water uptake as effected by the various treatments was obtained by re-weighing the sections at 48 hour intervals during the 6-day experimental period. The weighing was performed in a manner similar to that used initially.

RESULTS

CONTINUOUS INCUBATION OF DISCS: Data illustrating the effect of naphthaleneacetic acid (NAA)

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TABLE I

EFFECT OF VARIOUS CONCENTRATIONS OF NAA ON STIMULATION OF WATER UPTAKE IN POTATO DISCS

Treatment NAA	INITIAL FR WT	WATER UPT. 2	AKE PERIOD 4	(days) 6
(ppm)	(g)	INCREASE	IN FR WT	(%)
0.0*	1.11	6.3	10.8	12.6
0.01	1.11	5.4	11.7	13.5
0.1	1.12	4.5	17.0	21.4
1.0	1.10	6.4	32.7	49.1
10.0	1.11	8.1	37.8	55.9
50.0	1.11	0.9	10.8	14.4

* Distilled water control.

on the increase in fresh weight of potato tuber discs (table I) are very similar to those originally presented by Hackett and Thimann (12). A detectable stimulatory response is noted at a concentration of 0.1 ppm which increases in magnitude up to 10 ppm. It can generally be stated that a 0.01 g change in fresh weight is approximately equivalent to a one per cent change in this and subsequent tables. At 100 ppm toxic effects predominate (Data not illustrated). An important aspect of the data is that only small differences exist during the first 2 days of incubation. Later increases are much more pronounced.

The response of the discs to various concentrations of MH (table II) indicate that no pronounced inhibitory effects on the normal water uptake of the discs are noted until a concentration of approximately 20 to 50 ppm is reached. Thereafter, an increasing degree of inhibition ensues.

It is important to indicate here that all test solutions were aqueous and that the initial and final pH of the medium was not controlled on a routine basis. This was done for several reasons. First, it was found by buffer tests that the acidity of aqueous 100 ppm MH solutions (pH 4.9) was not a factor in the inhibition of water uptake in these tests (table III). Second, it was felt that difficulties in the interpretation of the data would arise in such tests, since the discs were exposed to the buffer salts for a comparatively long period of time and thus might be expected to be variously utilized in metabolic processes. Third, microbial activity would become troublesome.

In table IV data are presented on the interraction of the two chemicals. The MH concentration was varied while the NAA concentration was held at 1 ppm. By comparison with pertinent data in tables I and II, it is seen that 100 ppm MH almost completely negates the stimulatory action of the NAA. The lower concentrations of MH that were tested were ineffective in reducing the stimulatory action.

Data in table V describe the influence on water uptake of 100 ppm MH in combination with a series of NAA concentrations. It is shown that the stimulatory effects of NAA can be greatly or entirely suppressed. Again, pertinent data in tables I and II may be used for comparison.

TABLE II

EFFECT OF VARIOUS CONCENTRATIONS OF MH ON INHIBITION OF WATER UPTAKE IN POTATO DISCS

Treatment MH	INITIAL FR WT	WATER UP:	TAKE PERIO 4	D (days) 6
(ppm)	(g)	INCREASE	E IN FR W	r (%)
0.0*	1.13	6.2	13.3	14.2
0.01	1.11	5.4	12.6	15.3
0.1	1.12	6.3	13.4	15.2
1.0	1.11	5.4	12.6	14.4
10.0	1.11	7.2	13.5	15.3
20.0	1.12	6.3	10.7	11.6
50.0	1.10	5.5	9.1	10.0
100.0	1.13	5.3	8.0	8.8
500.0	1.13	2.7	6.2	3.5
1.000.0	1.10	-9.1	-37.2	

* Distilled water control.

PRETREATMENT OF DISCS: The foregoing data have been concerned with continuous exposure of the potato discs to various solutions of NAA and MH. The solutions supplied initially were never altered during the 6 day experimental period. While these data are important, they reveal very little about direct interraction phenomena between auxin-type growth

TABLE III

EFFECT OF BUFFERED & UNBUFFERED SOLUTIONS WITH & WITHOUT MH ON WATER UPTAKE IN POTATO DISCS

Treatment	Initial FR WT		ER UPTAKE PERIOD (4	days) 6
	(g)	Increase in FR wt ($\%$)		
Distilled water control at pH 6.9	1.12	7.1	11.6	13.4
100 ppm MH in water at pH 4.9	1.13	5.3	8.0	8.8
0.01 м pH 5 PO ₄ buffer	1.11	6.3	11.7	14.4
100 ppm MH in pH 5 PO ₄ buffer	1.12	5.4	8.0	8.9

TABLE IV EFFECTIVENESS OF VARIOUS CONCENTRATIONS OF MH ON DEPRESSION OF STIMULATORY ACTION OF 1 ppm NAA*

TREAT	MENT NAA	Initial Fr WT	WATER UPT	ake period 4	(days) 6
(pp	om)	(g)	INCREASE	IN FR WT	(%)
0.0	0.0**	1.13	6.2	11.5	12.4
0.01	1.0	1.12	8.9	36.6	51.8
0.1	1.0	1.14	7.0	38.6	51.7
1.0	1.0	1.12	11.6	42.9	56.3
10.0	1.0	1.15	8.7	38.3	46.1
100.0	1.0	1.15	5.2	13.9	17.4

^{*} As measured by water uptake in potato discs.

** Distilled water control.

stimulants and MH. A more direct approach to this problem is the pretreatment of the potato discs with the auxin followed by treatment with MH to study its influence on the residual auxin-induced water uptake. Conversely, pretreatments with MH, followed by treatments with NAA are of interest. Similar techniques have been used by workers in studies on auxin action (5).

A slight modification of the experimental procedure as described for continuous incubation of discs was required. The sections were weighed initially,

TABLE V EFFECTIVENESS OF VARIOUS CONCENTRATIONS OF NAA ON OVERCOMING INHIBITORY ACTION OF 100 ppm MH*

TREAT	iment —MH	INITIAL FR WT	Water upt	AKE PERIO 4	od (days) 6
(pi	om)	(g)	INCREASE	IN FR W	т (%)
0.0	0.0**	1.16	4.3	12.9	13.8
0.01	100	1.15	4.3	10.4	12.2
0.1	100	1.16	5.2	11.2	12.1
1.0	100	1.15	5.2	13.9	17.4
10.0	100	1.16	2.6	21.6	25.0

* As measured by water uptake in potato discs.

** Distilled water control.

incubated in the pretreatment medium for 48 hours, reweighed, transferred to the final solution, and weighed twice more at 48 hour intervals. Potato discs were thus pretreated with 1 ppm NAA and their subsequent response was observed after incubation in distilled water and several concentrations of MH (table VI). The total magnitude of the induced water uptake (24%) is less than if the sections were continuously incubated in NAA (49%). This increase is due entirely to the residual stimulatory effects of the NAA pretreatment. Similar phenomena have already been demonstrated (4). The present importance of these data lies in the inability of the

MH final treatment to reduce the residual stimulatory action of the NAA pretreatment. Thus, the inhibitory influence of MH on auxin induced water uptake is negligible once some auxin action has proceeded.

The converse effect, the influence of an MH pretreatment on the subsequent response of potato discs to NAA, was also studied (table VII). When used as a pretreatment of potato discs 100 ppm MH causes a considerable reduction in the subsequent water uptake that can be induced by NAA. Thus, the MH

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EFFECTIVENESS OF FINAL TREATMENTS WITH SEVERAL CONCENTRATIONS OF MH IN REDUCING RESIDUAL STIMULATORY ACTION OF WATER UPTAKE*

MH**	Initial fr wt	Water upt 2***	AKE PERIOD 4	(days) 6
(ppm)	(g)	INCREASE	IN FR WT	(%)
0.0+	1.10	9.1	20.0	23.6
1.0	1.07	7.5	18.7	23.4
10.0	1.08	9.3	18.5	23.1
100.0	1.09	10.0	18.3	22.0

* Induced a pretreatment of potato discs with 1 ppm NAA.

** Presented 2nd through 6th day.

*** End of 1 ppm NAA presentation. Discs immediately transferred to MH solutions.

+ Distilled water.

pretreatment reduces the sensitivity of the potato discs to the action of subsequently applied NAA.

Experiments involving the inclusion of mannitol in the pretreatment medium have also been performed. Such techniques allow water uptake to be separated from the predisposing cellular alterations induced by auxins, which cannot be demonstrated with just aqueous solutions. An 0.2 M mannitol solution is sufficient to deter water uptake of the potato discs

TABLE VII

EFFECTIVENESS OF 1 ppm NAA FINAL TREATMENTS IN OVERCOMING ACTION OF SEVERAL CONCENTRATIONS OF MH USED AS PRETREATMENTS

MH*	INITIAL FR WT	Water upt 2**	AKE PERIOR 4	(days) 6
(ppm)	(g)	INCREASE	IN FR WT	(%)
0.0***	1.15	6.1	13.9	22.6
1.0	1.16	6.9	15.5	25.0
10.0	1.14	6.1	13.2	21.1
100.0	1.10	4.5	9.1	11.8

* Presented for 2 days, from initial weighing to 2nd

day. ** End of MH presentation. Discs immediately trans-ferred to 1 ppm NAA solutions.

*** Distilled water.

TABLE VIII

EFFECT OF PR	ETREATMENTS	OF NAA	\ &/or M]	Н им 0.2 м
MANNITOL	ON SUBSEQU	JENT POI	CATO DISC	WATER
UPTAK	E IN VARIOU	's Aqueo	us Soluti	ONS

Treatmen pre* F	r (ppm) Sinal	Initial	WATE	r uptake (days)	PERIOD
0.2 м		fr wt	2**	4	6
MANNITOL Plus:		(g)	INCREA	SE IN FR	WT (%)
***	H.,O	1.20	0.8	15.0	19.2
10 NAA	H.,O	1.20	1.7	27.5	39.2
10 NAA	100 MH	1.19	1.7	20.2	31.1
100 MH	H.,O	1.19	2.5	13.4	21.0
100 MH	10 NAA	1.20	2.5	10.8	20.0

* Presented for 2 days, from initial weighing to 2nd

day. ** End of pretreatment presentation. Discs immediately transferred to final solutions indicated.

*** 0.2 м Mannitol only.

during the pretreatment period (table VIII). When NAA is included in the medium and is used as a pretreatment, apparently considerable auxin action proceeds in the presence of mannitol, even though water uptake is negligible in the first 48 hours. The auxin stimulation becomes evident after the discs have been placed in distilled water. The inclusion of MH in the final medium does not nullify the residual action of the NAA. These results resemble those given previously for NAA or MH pretreatments with no mannitol.

NATURE OF THE PROLONGED INHIBITION: Further investigations of the MH inhibitory phenomenon were attempted. The first phase involved the use of solutions of several inorganic salts immediately following pretreatment of the potato discs with MH to determine whether or not the inhibitory effects could be lessened by various ions active in physiological processes. The discs were pretreated with 100 ppm MH, immersed in the salt solutions for periods up to one hour, and then placed in 1 ppm NAA in an attempt to detect any lessening of the MH inhibitory influence. A 1 hour immersion in 1 \times 10⁻³ M KCl was slightly effective. Other salts were practically without effect.

The second phase of the study concerning the nature of the prolonged MH induced inhibition in water uptake of potato discs involved the utilization of C¹⁴labelled MH⁴. The object of these studies was to determine whether or not the MH pretreated tissue retained the MH at the end of the 6 day experimental period. The basic test procedure was that used in the water uptake experiments with the exception that 200 lambda of a radioactive 1 % solution of the diethanolamine salt of MH (specific activity 0.1 mC/

mm) was added to 30 ml of aqueous medium containing a total of 200 ppm MH. Weighed discs were incubated on this medium for the treatment period and then removed, blotted, weighed, and immediately ground for 2 to 3 minutes in a mortar and pestle containing 2 ml of water. This material was washed quantitatively into a graduated centrifuge tube and the final liquid volume recorded. This was usually less than 10 ml. The material was centrifuged at a slow speed for 10 minutes to separate the ground residue from the supernatant liquid. A 1 ml aliquot of the supernatant was withdrawn, evaporated, and counted. The remaining supernatant was removed until a total volume of 2 ml of supernatant plus residue remained in the tube. The residue was then resuspended in this 2 ml volume by agitation and a 1 ml aliquot placed on a planchet for counting. The counts for many samples were quite low, often being only twice background. A thin window G-M tube was utilized throughout. Residue samples were necessarily counted at infinite thickness. All data have been corrected for background.

The discs were pretreated with radioactive MH for 2 days with a subsequent transfer to final treatments of either distilled water or 1 ppm NAA for 4 days. Discs were sampled at 48 hour intervals for 6 days after the experiment had begun. This experiment was repeated. Data of a typical experiment (table IX) show that under conditions of pretreatment with MH, radioactivity remained within the sections for the entire 6 day period. Further, a considerable amount of the activity remained in the residue fraction, which also showed a consistent increase in activity over the experimental period. Incomplete grinding could not account for this effect. Also, at-

TABLE IX

PERSISTENCE OF C14-LABELLED MH IN POTATO DISCS EXPOSED TO A 2 DAY PRETREATMENT IN THE RADIOACTIVE SOLUTIONS OF MH*

Final	Fraction	Days 2**	5 OF SAMI 4	pling 6
MEDIUM		(cpm/	ml/g fr v	
	Supernatant	397	267	217
$H_{2}O$	Residue	316	491	422
	Supernatant	334	205	211
ppm NAA	Residue	359	364	472

* After pretreatment, the sections were immediately placed in either H_aO or 1 ppm NAA for 4 days. Sample discs separated by grinding and centrifuging into a supernatant and residue fraction at each sampling day.

** End of pretreatment period.

*** cpm/ml/g fr wt. Sample fresh weight in grams and the final total sample volume in ml were expressed as g/ml. This value was the same for both the supernatant and residue fractions of a given sample. Final figure derived by dividing g/ml figure into observed count rate previously corrected for background.

⁴ Kindly supplied by Naugatuck Chemical Corp., Bethany, Conn.

tempts to remove the activity from the residue with hot ethanol and hot 0.1 N HCl were only slightly effective. This suggests that the MH is held or incorporated into some component of the residue of the potato discs. Radioautographs prepared from chromatograms of the supernatant fractions which were developed in 8:1:1 isopropanol, ammonia, and water, showed the presence of MH at the end of the 6-day experimental period.

DISCUSSION

Interractions in in vitro systems between auxins and MH have been the subject of several previous studies (7, 8, 13, 14). Leopold and Klein first suggested that MH be considered as an antiauxin. Their hypothesis was based on results obtained in several of the auxin bioassay tests. They have reported that the inhibitory action of MH on cell elongation may be completely overcome by auxins and inversely that the inhibitory effects of high auxin concentrations may be overcome by MH. The latter effect has been questioned by Foster (7) who could find no such action. Foster stated that MH was a disproportionate inhibitor in the presence of low concentrations of auxins and that the effect of MH in the presence of high auxin concentrations observed by Leopold must have been the result of some characteristic of the assay solutions. More recently, work by Pilet (16) and Kulescha (13) suggests that the effect of MH is not on the levels of auxins, but rather on their utilization, and therefore the action of MH on auxin systems is best described as that of an auxin antagonist. These effects are displayed as an antagonism of one chemical on the expression of the particular stimulatory or inhibitory reaction induced by the other. Experiments related to those discussed above have been carried out in the present work to obtain more information on the nature of the interractions of MH and auxins. The experiments were concerned with the effects of MH and NAA alone and in combination on water uptake in potato discs. Continuous incubation of the discs indicates that at certain levels of concentration NAA is stimulatory to water uptake and MH is inhibitory. This is in agreement with previously published data from related tests (1, 14). When the two compounds are mixed at appropriate levels, the interractions produced show clearly that the MH inhibition is the dominant influence of the system and that the MH inhibition reduces the stimulatory effects of the NAA on cell enlargement. Its inhibitory influences on water uptake in the absence of added NAA are small and could possibly arise through a secondary effect on a process unrelated to auxin utilization. The question of the role of cell division in these stimulative and inhibitory effects quite naturally arises. This process definitely occurs over the 6-day period (11) but its significance in regard to the total water uptake induced by treatments of 1 ppm NAA is probably of little importance. Thus, the inhibitory action of MH in this case does not seem to arise from its antimitotic effect.

It is of interest to consider briefly some of the basic aspects of auxin action as they relate to the possible effects MH might have on these processes. Evidence has been accumulating in recent years that the primary effect of auxins is on plant cell walls (5). It is generally agreed that the initial phases of auxin action are concerned with processes involving oxidative metabolism and that the result of these processes is a reduction of cell wall pressure (18). This initial phase of action can be separated in time from a later elongation phase. There are a variety of reactions occurring in the initial phase which auxins reportedly affect in a stimulatory manner (3, 9, 15, 19). What is more significant concerning these reactions is that they can occur prior to any determinable growth and once completed will allow elongation to proceed even in the absence of external auxin. Enlargement is accomplished by a subsequent uptake of water by a purely osmotic process governed by the previous loosening of the cell wall.

By the use of a 48-hour auxin pretreatment period, we have shown in this study that certain reactions have taken place in the potato discs during this period, which may later be displayed as an increase in water uptake in the absence of further auxin treatment. Likewise, when these reactions are allowed to proceed in potato discs for 48 hours, the induced water uptake which subsequently occurs cannot be greatly inhibited by treatments with MH. Only a slight diminution of the residual stimulatory effects induced by a pretreatment with NAA occur. It is suggested that this could be due to a secondary effect of MH which is independent of auxin activity. This effect of MH on auxin pretreated discs is in direct contrast to the much greater inhibitory activity of MH on water uptake when the two chemicals are mixed together and presented simultaneously. Therefore, MH is possibly antagonistic to some initial phase of the auxincontrolled processes leading to cellular expansion.

An important relationship to the above discussion exists in a consideration of the effect of pretreatments of MH on the subsequent response of the potato discs to treatments of NAA. Pretreatments of the discs in distilled water did not preclude a subsequent readily detectable stimulatory response to NAA. Thus, any processes occurring during these initial 48 hours in the absence of auxin, such as Hackett (12) has found to occur with the terminal oxidase systems, are not of themselves important factors to consider in the later response of the potato discs to NAA. However, it was shown that inclusion of MH in the pretreatment almost completely suppressed any stimulatory action exerted by a subsequent treatment of NAA in the absence of any external MH. This is evidence for the suppression by MH of a reaction involved in the initial phases of auxin action on cellular expansion, for the discs do not respond to auxin treatment.

The experiments utilizing mannitol in the pretreatment medium substantiate these data. Although negligible uptake of water occurred during the pretreatments, there were pronounced effects on subsequent water uptake by pretreating with either NAA or MH. MH, therefore, is not greatly affecting the processes that are directly related to osmotic water uptake but presumably has its effect on the reactions which occur prior to this water uptake.

An explanation of this residual inhibitory action of MH on cellular enlargement as affected by auxins would be of significance in the understanding of the nature of the inhibitory mechanism of MH and perhaps the nature of the auxin process itself since the MH molecule is unlike most auxin antagonists. Several exploratory experiments were carried out in the present work in an effort to gain further insight to these problems. An immediate question to be answered was whether it was the MH molecule itself within these potato discs that was causing the inhibition, or a derived complex molecule. Thus, experiments were carried out in an attempt to leach the MH pretreated discs with solutions of various inorganic salts. It was thought that if free or very loosely bound MH was causing the inhibition, these salts could possibly be exchanged for the MH in the cellular milieu and thus affect a reduction in the residual inhibitory action. However, if the MH were all tightly bound, no such reduction could result. Considering the possible stimulatory effects the various inorganic ions could have had on the potato discs. only slight relief in the residual inhibitory capacity was encountered, suggesting therefore, a possible binding of the MH to some cellular entity. The results of the experiments utilizing radioactive MH in the potato discs' incubation medium tend to confirm this supposition. Chromatographic results indicated that there was a certain amount of free MH in the discs for the duration of the experiment. Further, it was found that a considerable portion of the radioactivity within the discs was located on, and rather strongly affixed to, the solid material obtained on grinding the discs in distilled water. The data also indicate that the incorporation of radioactivity into the solid fraction increases with time.

Previous studies have indicated that much of the inhibitory action of MH is due to its effect on mitosis (6, 10). There are, however, several lines of evidence in addition to those presented in this study, which indicate that this supposition is not entirely correct. First, it has never been established that the anti-mitotic action of MH is a primary effect, thus allowing the assumption that its anti-mitotic action is a secondary effect due to the inhibition of a related process. This is entirely plausible when the variety of cellular reactions reported to be effected by MH are considered. Second, and perhaps most significant, is the fact that MH does not commonly show inhibitory activity in tissues other than those of higher plants (2). It seems possible then, that its influence could be exerted on some system which is unique to

higher plant tissues. One readily apparent difference of cells of higher plant origin lies in the cellulosic nature of the cell walls where, presumably, the initial phases of auxin action occur. Certain processes occurring during this period have been shown to be affected by MH in the experiments previously discussed. The recent discovery of Towers (20) that MH forms a glucose glycoside upon incubation of wheat leaves in a medium containing MH and glucose is of importance also. The finding in the present study that MH tends to accumulate in the solid fraction of potato discs is suggestive of an affinity for certain constituents of the cell wall. The residual inhibitory influence of MH to any subsequent auxin-induced cell enlargement further supports an assumption that its activity is associated with some component related to cellular expansion. Towers has suggested that the glycoside found in wheat leaves constitutes a detoxification mechanism of the plant. In view of what has been presented here concerning the nature of the MH-auxin interaction, it is interesting to speculate how this glycoside, or some product related to it might be inhibitory to the process of auxin-induced cell elongation. The results obtained in this study suggest that MH does exert an inhibitory influence on auxininduced cell enlargement, thus giving rise to the interactions heretofore demonstrated. Further evidence is required, however, to ascertain the significance of the apparent affinity of MH for cellular materials under the influence of added exogenous auxins.

SUMMARY

The influence of MH and NAA on water uptake in potato discs has been studied. Both continuous incubation and pretreatment of the discs has been employed. These compounds were presented separately, successively, and in mixtures of varied concentration. With these experimental conditions, it was found that under conditions of continuous incubation MH can negate the stimulatory action of NAA on water uptake. The NAA apparently cannot overcome this inhibition. By pretreating the discs with NAA and then exposing them to MH, the inhibitory action of the MH does not appear to be effective. Conversely, pretreating the discs with MH renders them insensitive to any subsequent stimulatory action of the NAA. It is suggested that in this case the MH is acting on a process related to auxin-induced cellular expansion.

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