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A STUDY OF THIOAMIDE-INDUCED GERMINATION OF SEEDS OF *PRUNUS PERSICA**

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Abstract—Thioacetamide, thiourea, and 1-allyl-2-thiourea were effective in promoting germination of non-after-ripened seeds of "Lovell" peach. The activity of thioamides in promoting germination of dormant peach seeds appears to be dependent on tautomerism of the thioamide to form the thiol-imido configuration with both the thiol and imido groups being necessary for activity. A marked interaction between thiourea and α-naphthaleneacetic acid suggested that thioamides influence germination by their effect on auxin activity. Maleic hydrazide and kinetin had no effect on germination either alone or in the presence of thiourea. Gibberellic acid and thioamides appear to influence different mechanisms in promoting germination of dormant peach seeds.

INTRODUCTION

PEACH seeds require a period of low temperature after-ripening before germination and normal seedling development will occur. Inasmuch as the embryos of peach seeds are not completely dormant, germination of non-after-ripened seeds may be induced by removal of the seed coats and associated tissues. In addition, the germination of non-after-ripened peach seeds may be forced by treating the intact seeds with thiourea[1–3] or gibberellic acid.[4–6]

Thiourea is perhaps one of the most interesting of the chemicals found to stimulate seed germination because of its promotive effect on both light- and cold-requiring seeds. Furthermore, this compound has been found to have pronounced effects on mitosis and morphogenesis in lower plants and animals. Thompson and Kosar[7] verified the importance of sulfur in the activity of thiourea and certain related chemicals as promoters of germination of freshly harvested, dormant lettuce seeds. Toole *et al.*[8] suggested that the activity of thiourea as a germination promoter depended on tautomerism of the thioamide to form the thiol-imido configuration, the derivative thus being functionally related to thiols such as cysteine and glutathione.

The following studies were made to determine the effects of thiourea and certain structurally similar compounds on the germination of non-after-ripened peach seeds (*Prunus persica* Batsch. cv. Lovell) which normally require 40–60 days of moist storage at 5° before germination will take place. In addition, studies were conducted of the interactions of thiourea with a number of other chemicals which have been reported to affect seed germination.

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RESULTS

Effect of Sulfur-containing Compounds on Seed Germination

A study was made of the effects of certain sulfur-containing chemicals on the germination of intact, non-after-ripened seeds. All of the compounds tested contained divalent sulfur with the exception of the oxygen analogs of thiourea and thioacetamide (Table 1). Samples, containing 20 seeds each, were soaked for a period of 10 hr in 10^{-3}, 10^{-2}, and 10^{-1} M solutions of each compound after which they were thoroughly washed and placed for germination in seed flats filled with moist vermiculite. In addition, compounds which had extremely low solubilities in water (*o*-mercaptobenzoic acid, thioacetanilide, and 2-mercaptoacetanilide) were applied to seeds in dilute (10^{-3} M) solutions throughout the germination period.

In order to determine whether or not non-germinated seeds in each treatment were injured by the treatments, seed coats and associated tissues were removed from the non-germinated seeds on the 16th day following exposure. These excized embryos were held for an additional 14-day period and their germination observed.

Thiourea, thioacetamide, and 1-allyl-2-thiourea were effective in causing germination of intact, non-after-ripened seeds, and of these three, thioacetamide and thiourea were considerably more effective than allylthiourea (Table 1). Moreover, in further tests, it appears that thioacetamide is a more effective compound in stimulating peach seed germination than thiourea. The oxygen analogs of thiourea and thioacetamide, urea and acetamide, did not stimulate germination under the conditions of these experiments. Furthermore, other compounds containing divalent sulfur did not promote germination of the intact, dormant seeds.

Although many of the substances had no effect in promoting germination of intact seeds, they apparently stimulated growth of the embryos that were subsequently excized from these seeds (Table 1). Compounds which stimulated growth of excized embryos included thiourea,

TABLE 1. EFFECT OF A NUMBER OF COMPOUNDS ON THE GERMINATION OF NON-AFTER-RIPENED "LOVELL" PEACH SEEDS

Compound	Concentration (M)	Percent germination	
		Intact	Excised[a]
Thiourea	10^{-1}	60	100
	10^{-2}	0	93
	10^{-3}	0	47
Urea	10^{-1}	0	93
	10^{-2}	0	81
	10^{-3}	0	50
Thioacetamide	10^{-1}	75	100
	10^{-2}	0	36
	10^{-3}	0	84
Acetamide	10^{-1}	0	75
	10^{-2}	0	31
	10^{-3}	0	28
Thiosemicarbazide	10^{-1}	5	63
	10^{-2}	0	70
	10^{-3}	0	83
Thiocarbohydrazide	10^{-1}	0	100
	10^{-2}	0	50
	10^{-3}	0	93
1-Allyl-2-thiourea	10^{-1}	15	80
	10^{-2}	5	62
	10^{-3}	5	65

TABLE 1—*cont.*

Compound	Concentration (M)	Percent germination Intact	Excised*
Thiobarbituric acid	10^{-1}	0	47
	10^{-2}	0	35
	10^{-3}	0	28
Thioacetic acid	10^{-1}	0	Dead
	10^{-2}	0	32
	10^{-3}	0	33
Mercaptoacetic acid	10^{-1}	0	Dead
	10^{-2}	0	27
	10^{-3}	0	50
Mercaptoethanol	10^{-1}	0	Dead
	10^{-2}	0	29
	10^{-3}	0	16
Mercaptoethylamine	10^{-1}	0	40
	10^{-2}	0	60
	10^{-3}	0	78
Mercaptosuccinic acid	10^{-1}	0	40
	10^{-2}	0	50
	10^{-3}	0	47
Sodium thiosulfate	10^{-1}	0	60
	10^{-2}	0	77
	10^{-3}	0	50
Ammonium thiocyanate	10^{-1}	0	80
	10^{-2}	0	50
	10^{-3}	0	80
Potassium thiocyanate	10^{-1}	0	50
	10^{-2}	0	50
	10^{-3}	0	23
Sodium thiocyanate	10^{-1}	0	56
	10^{-2}	0	56
	10^{-3}	0	64
Water controls 1		0	30
2		0	50

* Intact seeds were initially soaked for a period of 10 hr in solutions of the various compounds. If the seeds had not germinated by the 16th day after treatment, the seed coats were removed and the embryos were placed for germination in the dark at 20° without further treatment with the solutions. Germination was checked on the 7th day after excision (the 23rd day after the initial treatment of intact seeds.)

urea, thioacetamide, acetamide, thiosemicarbazide, thiocarbohydrazide, allylthiourea, mercaptoethylamine, sodium thiosulfate, and ammonium thiocyanate; those which apparently had no effect on the germination of subsequently excized embryos included thiobarbituric acid, mercaptosuccinic acid, potassium thiocyanate, and sodium thiocyanate. High concentrations (0·1 M) of thioacetic acid, mercaptoacetic acid, and mercaptoethanol caused death of the embryos while lower concentrations slightly inhibited germination or had no effect at all.

Compounds which were applied to intact seeds in dilute solutions throughout the germination period (*o*-mercaptobenzoic acid, thioacetanilide, and 2-mercaptoacetanilide) did not promote germination although similar treatment with thiourea resulted in 30 per cent seed germination.

Interaction Studies

Samples, each containing 10 fully imbibed peach seeds, were soaked for 6 hr in each of 6 concentrations of α-naphthaleneacetic acid, (NAA), maleic hydrazide, and kinetin in combination with 6 concentrations of thiourea. Each treatment was replicated 3 times. In studies with gibberellic acid (GA), samples containing 25 seeds each were used, and each treatment was replicated 4 times.

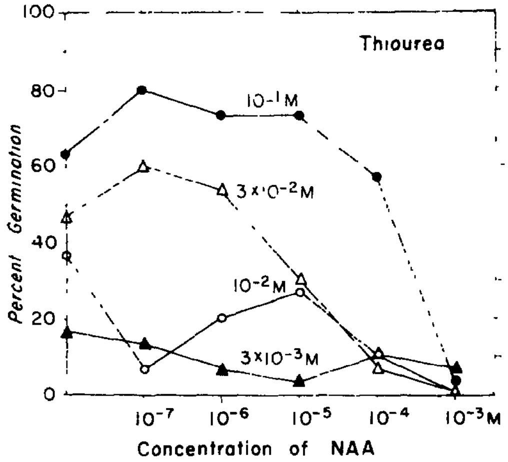


FIG. 1. THE EFFECT OF THIOUREA AND NAPHTHALENEACETIC ACID (NAA) ON THE GERMINATION OF NON-AFTER-RIPENED SEEDS OF "LOVELL" PEACH.

Seeds were soaked for 10 hr in the various solutions after which they were thoroughly washed in tap water and placed for germination in the dark at 20°.

TABLE 2. THE INTERACTION OF THIOUREA AND NAPHTHALENEACETIC ACID ON THE GERMINATION OF NON-AFTER-RIPENED SEEDS OF "LOVELL" PEACH*

Concentration of thiourea (M)	Per cent germination Concentration of naphthaleneacetic acid					
	0	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3} M
0	0	6·7	0	0	0	0
10^{-3}	0	0	0	0	3·3	0
3×10^{-3}	16·7	13·3	6·7	3·3†	10·0	6·7†
10^{-2}	36·7	6·7‡	20·0‡	26·7†	10·0‡	0‡
3×10^{-2}	46·7	60·0†	53·3	30·0‡	6·7‡	0‡
10^{-1}	63·3	80·0‡	73·3†	73·3†	56·7	3·3‡

* Thirty seeds (three replicates of 10 seeds each) were soaked for 6 hr in the various combinations of thiourea with naphthaleneacetic acid. Germination was observed on the 14th day after treatment.
† Significant at the 5 per cent level for the action of NAA as determined by the F-test
‡ Significant at the 1 per cent level.

The effects of NAA, alone and in combination with thiourea, on the germination of intact, non-after-ripened seeds are shown in Table 2 and Fig. 1. When used either alone or with concentrations of thiourea which did not promote germination (10^{-3} M), NAA was ineffective in stimulating germination at any of the various concentrations tested. If, however, the thiourea concentration were adjusted to give low percentages of germination (3×10^{-3} and 10^{-2} M), NAA-inhibition of seed germination was observed. Furthermore, if the thiourea treatment resulted in germination percentages of approximately 50 per cent or higher, NAA either promoted or inhibited germination depending on its concentration. In the presence of 3×10^{-2} M thiourea, NAA stimulated germination at a concentration of 10^{-7} M but inhibited germination at 10^{-5}, 10^{-4}, and 10^{-3} M concentrations. In the presence of a greater amount of thiourea (0·1 M), promotion of germination by NAA was observed at 10^{-7}, 10^{-6}, and 10^{-5} M concentrations of the auxin while inhibition occurred at 10^{-3} M concentration. Thus, as the concentration of thiourea was increased, the concentrations of NAA for both promotion and inhibition of germination were shifted to higher values.

Gibberellic acid (GA) stimulated germination of non-after-ripened seeds both in the presence and absence of thiourea (Table 3). With the exception of minor variations, these

TABLE 3. THE EFFECT OF THIOUREA AND GIBBERELLIC ACID ON THE GERMINATION OF NON-AFTER-RIPENED SEEDS OF "LOVELL" PEACH*

Concentration of thiourea (M)	Per cent germination Concentration of gibberellic acid					
	0	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2} M
0	0	0	0	11‡	7†	23‡
10^{-3}	0	3	0	9†	13‡	19‡
3×10^{-3}	6	0	0	15†	3	10
10^{-2}	7	11	2	21‡	26‡	29‡
3×10^{-2}	20	14	27†	21	38‡	53‡
10^{-1}	42	39	44	49†	55‡	73‡
3×10^{-1}	66	72	62	71†	78‡	86‡

* One hundred seeds (four replicates of 25 seeds each) were soaked for 6 hr in the various combinations of thiourea and gibberellic acid. Germination was observed on the 14th day after treatment.

† Significant at the 5 per cent level for the action of gibberellic acid as determined by the F-test.

‡ Significant at the 1 per cent level.

data suggest that thiourea and gibberellic acid act independently in promoting peach seed germination, and that the effects of these chemicals are additive. This additive response was most apparent with treatments which gave high percentages of germination.

Observations made during germination of seeds treated with thiourea and GA suggested that these chemicals induce two distinct types of germination response. In the case of thiourea-induced germination, there was no visible enlargement of cotyledons during sprouting of the seeds, and the first evidence of growth was the appearance of rapidly elongating radicles as they pierced the seed coats. In the case of GA-induced germination, initial enlargement of the cotyledons was pronounced, and increases in embryo volume appeared to cause rupture of the seed coats prior to any radicle growth. It is possible, therefore, that radicle elongation in this instance resulted from separation of the growing points from the enveloping seed coats in much the same manner as embryo excision. The promotion of germination by mechanical means (the rupture of seed coats by increases in embryo volume) as a result of treatment with GA would explain much of the variability in germination observed during this study.

In similar tests, maleic hydrazide and kinetin were found to have no promotive effect on germination when the chemicals were applied individually to the seeds. Furthermore, these compounds had no effect in either enhancing or inhibiting thiourea-induced germination.

DISCUSSION

While these studies support the previous observation[7] that organic sulphur is essential to the activity of thioamides in promoting seed germination, this activity does not appear to depend solely on the presence of a free sulfhydryl group in the thioamide molecule. If this were the case, other thiols might also be expected to stimulate germination. Treatments with glutathione (unpublished), thioacetic acid, mercaptoacetic acid, mercaptoethanol, mercapto-ethylamine, and mercaptosuccinic acid were without effect in promoting germination of intact peach seeds. The fact that all of these compounds influenced (promoted or inhibited) germination of the embryos subsequently excized from the treated seeds is evidence that these agents did reach potentially active sites but were unable to overcome the existing block to growth.

Tautomerism of thiomides results from: (1) electromeric displacement to form an anionic center at the sulfur atom, (2) the separation of a proton from the amine group as a result of increasing acidity, (3) a rearrangement of electrons of the nitrogen atom to form an imido group, and (4) the recombination of the disengaged proton at the sulfur atom (Fig. 2).

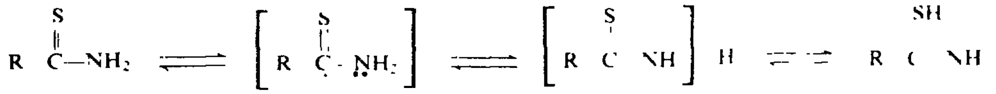
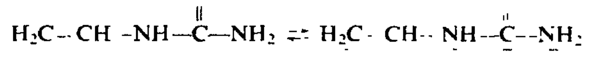


FIG. 2. TAUTOMERISM OF THIOAMIDES

Electron enrichment of the sulfur-bearing carbon atom resulting from the release of an unshared electron pair by the nitrogen atom facilitates the establishment of an anionic center at the sulfur atom. Therefore, any substitution on the sulfur-bearing carbon atom that influences electromeric displacement to form this anion would also influence the tautomeric equilibrium. Thiourea (Fig. 2, R = $-NH_2$) contains two primary amine groups, each equally capable of contributing to the molecular rearrangement. Which group participates in the tautomerism is of no consequence and is based wholly on chance.

While thiourea was found to be quite effective in promoting germination, thioacetamide (Fig. 2, R = $-CH_3$) was found to be more effective at the same concentration. It is believed that the increased activity of thioacetamide results from the electron-releasing properties of the methyl group. Electron release by this group with the accompanying electron enrichment of the sulfur-bearing carbon atom facilitates anion formation and shifts the tautomeric equilibrium toward the thiol-imido form.

The N-substitution of thiourea with the vinyl group to form allylthiourea markedly reduced activity in promoting germination. This reduction in activity may result from the tendency of the vinyl group to polarize in aqueous solutions. Polarization results in electron displacement to form a condition of alternating partial charges involving the vinyl group, the substituted amine group, the sulfur-bearing carbon atom, and the primary amine group (I↔II). Under these conditions, the reduction of electron density around the sulfur-bearing



II

carbon atom does not favor the establishment of the required anion, and the tautomeric equilibrium is shifted away from the thiol-imido form. The N-substitution of thiourea with another amine group (thiosemicarbazide) and the N,N′-substitution with two amine groups (thiocarbohydrazide) reduced activity 91 per cent in the former case and completely eliminated activity in the latter case. Decreases in activity resulting from these substitutions may also be attributed to their influence on anion formation. It appears, therefore, that the activity of thioamides in promoting seed germination is directly dependent on the capacity of these compounds to form thiol-imido tautomers. The fact that urea and acetamide, compounds which have no effect in promoting germination, also tautomerize to give imido forms is evidence that this group alone does not satisfy the specific requirements of a germination promoter and further suggests that both the sulfhydryl and imido groups are requisite to activity in promoting germination. While this information does not suggest the mode of action of thioamides in stimulating growth of dormant seeds, recognition of essential chemical groups may provide clues to mechanisms involved in the action of these agents.

Seed dormancy, according to certain current views, may result from either the lack of essential growth promoting substances or the presence of active growth inhibitors in the tissues. In view of the lack of response of dormant peach seeds to treatment with NAA, it appears unlikely that seed dormancy in this case results from a suboptimal concentration of auxin. Furthermore, there is little reason to believe that thiourea is effective in promoting germination by increasing endogenous auxin levels. Studies of the interaction of thiourea and NAA suggest that enhancement of germination by NAA may be related to the capacity of the thioamide to remove an endogenous block to growth, thereby allowing the tissue to respond to exogenously applied auxin. An analogous situation has been reported by Gouwentak[9] who found that indoleacetic acid stimulated growth of dormant cambial cells in *Fraxinus* twigs only if the rest period had been previously broken by treatment with ethylene chlorohydrin. In this case, auxin was believed to supply only the necessary growth factor for cells which had been previously rendered capable of growth. Chauhan[6] reported that NAA stimulated the germination of dormant "Lovell" peach seeds, but found the auxin to be considerably more effective if the seeds were partially after-ripened prior to treatment with the compound. It appears, therefore, that certain specific changes must take place in dormant peach seeds before auxins, native or applied, are capable of influencing growth, and that these changes may be brought about by after-ripening treatments, embryo excision, or treatment with certain thioamides. While this study provides no direct information as to the nature of the block to germination, the fact that thiourea has been shown to counteract the effects of a number of naturally occurring germination inhibitors[10–12] may be considered indirect evidence that this compound has a similar role in promoting germination of dormant peach seeds.

Comparatively recent evidence for the occurrence of active gibberellins in a number of seeds requires consideration of the possibility that the activity of thioamides in promoting germination results from their effect on the levels and/or activity of native gibberellins. It is interesting to note that Chauhan[6] found the activity of GA in promoting germination of dormant "Lovell" peach seeds to be reduced when the chemical was used in conjunction with maleic hydrazide. During these studies, maleic hydrazide was found to have no effect on thiourea-induced germination. This observation along with the previously-mentioned differences in germination responses suggest that thioamides and GA have different mechanisms of action in promoting germination.

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In a recently reported study, Bruce *et al.*[13] found that a number of derivatives of both urea and thiourea affected a variety of biological systems in a manner typical of phytokinins. These authors state that these derivatives should be classed as kinins because of their activities in stimulating cell division, retarding senescence, promoting bud development, and/or stimulating seed germination. It is interesting to note that the compounds reported to have activity in the test systems used by these authors cannot fulfill the structural requirements reported from this investigation to be necessary for peach seed germination. That the germination of peach seeds is not a kinin-induced response is also supported by the observation made during this study that kinetin (6-furfurylaminopurine) had no effect in promoting germination of these seeds, either alone or in the presence of thiourea.

EXPERIMENTAL

Plant Material

Non-after-ripened peach seeds of the "Lovell" variety were removed from the stony endocarps, carefully graded, and stored in glass containers at laboratory temperatures until they were used.

Treatments

While periods of exposure of the seeds to the various chemicals were varied from experiment to experiment, a number of procedures were standard throughout this study. All seeds were placed in moist vermiculite at 20° for a 48-hr-period of imbibition prior to chemical treatment. All treatments provided 1·0 ml of chemical solution for each seed in the sample. Treatments were conducted in the dark at 20° after which the seeds were placed in metal seed flats containing moist vermiculite for germination in the dark at 20°. Additional water was added to the flats as required.

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