disorder was always prevalent. Also, it did not occur as frequently on shoots from axillary buds, even when grown in seed flats where the condition was widespread. Differences observed in the frequency of tip necrosis on light-grown and dark-grown seedlings may indicate that light plays a major role in preventing this disorder.

SUMMARY

Observations made of seedlings developing from non-afterripened seeds of 'Lovell' peach showed that, irrespective of treatment to induce germination or conditions under which seeds were held during germination, the seedlings developed abnormally. Abnormal development was usually restricted to epicotyls or, in some cases, to shoots from axillary buds. It was characterized by failure of internodes to elongate and, in many instances, by failure of leaves to develop normally. Exposure of seeds to biologically high temperatures caused a marked increase in the severity of abnormal epicotyl development. Moist storage of seeds for extended periods at 20° to 25° C. prior to embryo excision inhibited radicle elongation during the early stages of germination and, in many cases, completely inhibited epicotyl elongation. Dieback of plumules was found to occur frequently in large portions of seedling populations. Root systems of seedlings developing from non-afterripened seeds were apparently normal.

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INFLUENCE OF GIBBERELLIC ACID, MERCAPTOETHANOL, MERCAPTOETHYLAMINE, THIOUREA, AND UREA ON THE GERMINATION OF 'OKINAWA' PEACH SEEDS[1]

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INTRODUCTION

Gibberellic acid and thiourea have been found to be effective in promoting germination of dormant seeds of certain peach cultivars (2, 5, 6, 9). However, seedlings produced from dormant seeds by thiourea treatments often develop abnormally (6, 9) and resemble those grown from inadequately afterripened seeds (1, 2, 4, 7, 8).

From his studies on the effects of gibberellic acid, thiourea, and a number of thiourea derivatives on the germination of dormant 'Lovell' peach seeds, Garrard (6) suggested that gibberellic acid and thioamides promoted peach seed germination by different mechanisms. In connection with the effects of thioamides on germination, he suggested that the activity of this group of compounds appeared to depend on tautomerism to form the thioimido configuration. Both the thiol and imido groups were thought to be necessary for activity. However, it was not determined whether or not these two structures were required on the same compound.

The purpose of the experiment reported in this paper was to determine whether non-after-

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ripened peach seeds could be forced to germinate and produce normal seedlings by providing the thiol and imido groups simultaneously but each as a part of a separate compound. Concurrently, to form a basis for comparison, 'Okinawa' peach seeds were tested as to their response to thiourea and gibberellic acid, respectively.

Materials and Methods

Seeds of *Prunus persica* cv. 'Okinawa,' used in this study, were of the 1962 crop harvested from the Horticultural Unit of the Agricultural Experiment Station, Gainesville, Florida. Fruits were collected, pits cleaned and dried, and seeds removed from the endocarp in accordance with good horticultural practices. Then, the seeds were stored in a dry place at 22° to 26° C. until they were used.

Chemicals used in this investigation included gibberellic acid, thiourea, 2-mercaptoethanol, 2-mercaptoethylamine, and urea. Gibberellic acid was an 80 per cent formulation that was graciously supplied by the Abbott Laboratories, North Chicago, Illinois. The other chemicals were of the highest purity that could be obtained commercially.

The pretreatment of the seeds, prior to chemical treatment, was the same for all tests. Seeds were planted in moist vermiculite and kept at 20° ± 1° C. for 48 hours. Then, the seeds were washed to remove the adhering vermiculite, blotted, and placed in an aqueous medium, with or without the chemical addendum. Fluid volumes and containers were chosen so that there was 1 ml. of solution per seed, and the seeds were barely covered with the solution to avoid anaerobic conditions. After a 6-hour soaking period, seeds were removed from the solutions, blotted, and planted in 4-inch plastic pots, containing a 1:1 mixture of soil and perlite. Then, they were placed in the dark at 20° ± 1° C. in a walk-in germinator. After 14 days, the seeds were checked for germination and the germinated seeds transferred to a growth chamber kept at 21° ± 1° C. with a 12-hour day and night regime. The light intensity at the surface of the soil was approximately 900 ft-c. The remaining seeds that had not germinated were subjected to embryo excision to induce germination and returned to the darkened germinator for an additional 14 days before they were transferred to the 12-hour photoperiod regime. Daily observations were made of the seedlings for the appearance of growth anomalies until they had developed at least 12 mature leaves. Statistical methods used were suggested by Dr. A. E. Brandt, Department of Statistics, University of Florida. They included the "analyses of variance" for the influence of each chemical on seed germination and "factorial chi square" for interactions of chemicals on seed germination.

Experimental Results

Effect of chemicals on seed germination—Thiourea had a marked promotive effect on the germination of 'Okinawa' seeds. As can be seen in Table 1, all 6 concentrations of thiourea tested produced statistically significant increases in seed germination. The 10^{-1} M concentration of the chemical caused 100 per cent germination in two of the replications, and 90 per cent in the third.

Gibberellic acid induced less 'Okinawa' seed germination than thiourea. This can be seen by comparing Tables 1 and 2. Due to the lesser increases in seed germination induced by gibberellic acid, the small seed samples, and the variability between replicates, the F-test was almost significant at the 5 per cent level for increases in germination. This support, coupled with subsequent tests of gibberellic acid, would seem to indicate that the chemical does indeed increase 'Okinawa' seed germination.

Neither mercaptoethanol, mercaptoethylamine, nor urea had any significant effect on the germination of 'Okinawa' seeds. This can be seen from the data recorded in Tables 3, 4 and 5.

In an attempt to promote seed germination by

Table 1

Effect of Thiourea on the Germination of 'Okinawa' Peach Seeds (10 Seeds per Replication).

Code	Concentration of Thiourea	Number of Seeds Germinated[1] Replication 1	2	3
A	3×10^{-1} M	6	6	9
B	10^{-1}	10	10	9
C	3×10^{-2}	5	6	6
D	10^{-2}	6	5	4
E	3×10^{-3}	2	5	3
F	10^{-3}	2	3	4
G	0	1	1	0

Source of Variation	D/F	SS	MSS	F
Treatment	6	155.14	25.86	20.09 **
Replication	2	1.23	0.61	.47 NS
Error	12	15.44	1.29	
Total	20	171.81		

Duncan's New Multiple Range Test (3)

B A C D E F G

[1]Seed germination checked on the 14th day after chemical treatment.

Table 2

Effect of Gibberellic Acid on the Germination of 'Okinawa' Peach Seeds (10 Seeds per Replication)

Code	Concentration of Gibberellic Acid	Number of Seeds Germinated[1] Replication 1	2	3
A	3×10^{-1} M	3	3	5
B	10^{-1}	7	4	2
C	3×10^{-2}	2	3	5
D	10^{-2}	5	2	3
E	3×10^{-3}	2	1	1
F	10^{-3}	2	4	1
G	0	0	0	0

Source of Variation	D/F	SS	MSS	F
Treatment	6	40.95	6.82	2.88 NS[2]
Replication	2	1.52	.76	.32
Error	12	28.48	2.37	
Total	20	70.95		

[1]Seed germination checked on the 14th day after treatment.

[2]To be significant at the 5 per cent level, 3.00 was needed.

Table 3

The Influence of Mercaptoethanol on Germination of 'Okinawa' Peach Seeds (10 Seeds per Replication).

Concentration of Mercaptoethanol	Number of Seeds Germinated[1] Replication 1	2	3
10^{-1} M	0	0	1
3×10^{-2}	0	1	1
10^{-2}	2	2	1
3×10^{-3}	2	1	3
10^{-3}	2	1	2
10^{-4}	0	1	0
0	1	1	1

Source of Variation	D/F	SS	MSS	F
Treatment	6	8.18	1.36	.98 NS
Replication	2	.38	.19	.14 NS
Error	12	16.63	1.38	
Total	20			

[1]Seed germination checked on the 14th day after chemical treatment.

Table 4

Influence of Mercaptoethylamine on the Germination of 'Okinawa' Peach Seeds (10 Seeds per Replication)

Concentration of Mercaptoethylamine	Number of Seeds Germinated[1] Replication 1	2	3
10^{-1} M	0	2	2
3×10^{-2}	0	1	0
10^{-2}	1	0	1
3×10^{-3}	2	0	3
10^{-3}	2	2	0
10^{-4}	1	2	0
0	1	1	0

Source of Variation	D/F	SS	MSS	F
Treatment	6	5.70	.95	1.25 NS
Replication	2	2.14	1.07	1.40 NS
Error	12	9.12	.76	
Total	20	16.96		

[1]Seed germination checked on the 14th day after chemical treatment.

treating seeds with a mixture of a thiol-containing compound and an amide-containing compound capable of forming an imido group, mercaptoethanol and urea were applied to seeds in various combinations of concentration between the two chemicals. As can be seen in Table 6, the only significant change in the pattern was due to an inhibition of germination by the 10^{-1} M concentration of mercaptoethanol, alone or in combination with the 10^{-1}, 10^{-2}, or 10^{-3} M concentration of urea. This inhibition of germination was statistically significant at the 5 per cent level.

Effect of chemicals on seedling development—Since thiourea and gibberellic acid were the only chemicals that stimulated the germination of intact seeds to any extent, only data for the influence of these two chemicals on normal vs. abnormal seedlings from intact seeds can be pre-

Table 5

Effect of Urea on the Germination of 'Okinawa' Peach Seeds (10 Seeds per Replication).

Concentration of Urea	Number of Seeds Germinated[1] Replication 1	2	3
3×10^{-1} M	2	1	0
10^{-1}	3	1	0
3×10^{-2}	1	2	2
10^{-2}	2	2	1
3×10^{-3}	2	1	2
10^{-3}	0	0	1
0	2	1	0

Source of Variation	D/F	SS	MSS	F
Treatment	6	4.47	.745	1.03 NS
Replication	2	2.67	1.33	1.85 NS
Error	12	8.67	.72	
Total	20	15.81		

[1]Seed germination checked on the 14th day after chemical treatment.

Table 6

Effect of Urea and Mercaptoethanol and Combinations of the Two Chemicals on the Germination of 'Okinawa' Seeds (30 Seeds per Treatment).

Concentration of Urea	Number of Seeds Germinated[1] Concentration of Mercaptoethanol 10^{-1} M	10^{-2} M	10^{-3} M	0
10^{-1} M	0	2	3	4
10^{-2}	1	2	3	5
10^{-3}	0	3	3	1
0	1	5	5	3

Source of Variation	D/F	SS	Chi Square Factor	Chi Factor
Mercaptoethanol	3	0.6223	14.056	8.747 *
Urea	3	.2060	14.056	2.895 NS
Mercaptoethanol and Urea	9	.4959	14.056	6.970 NS

[1]Seed germination checked on the 14th day after chemical treatment.

sented. It is apparent from Table 7 that abnormal seedling development, characterized by the occurrence of misshapen and malformed leaves, was found to be prevalent among seedlings from seeds which germinated as a result of the thiourea treatment. In contrast to this, seedlings from seeds treated with gibberellic acid exhibited very few growth anomalies.

Among seedlings from all of the various chemical treatments that failed to germinate the first 14 days, but were subsequently induced to grow by removing the seed coat and associated tissues, there were very few that had any growth anomalies.

Discussion

That both gibberellic acid and thiourea were found to stimulate germination of dormant 'Okinawa' peach seeds would seem to indicate that the mechanism controlling germination in this low-chilling cultivar of peach, that requires short periods of stratification for the resumption of growth, is similar to that controlling germination in other cultivars which require much longer periods of low-temperature stratification.

Thiourea was found to be particularly effective in promoting the germination of dormant 'Okinawa' peach seeds, while its oxygen analog, urea, was not effective in this regard. In addition, the thiols, mercaptoethanol and mercaptoethylamine, did not increase germination. These findings are in agreement with those previously reported for seeds of 'Lovell' peach (6).

Table 7

The Influence of Thiourea and Gibberellic Acid on Germination of 'Okinawa' Peach Seeds and on Anomalous Growth of the Seedlings (30 Seeds per Treatment)

Treatment: Chemical and Concentration	Percentage of Seeds Germinated[1]	Percentage of Seedlings Exhibiting Growth Anomalies[2]
Thiourea, 3×10^{-1} M	70	52
" 10^{-1}	97	62
" 3×10^{-2}	57	47
" 10^{-2}	50	40
" 3×10^{-3}	33	30
" 10^{-3}	30	44
Gibberellic Acid 3×10^{-1} M	37	9
" 10^{-1}	43	0
" 3×10^{-2}	33	10
" 10^{-2}	33	0
" 3×10^{-3}	13	0
" 10^{-3}	23	0
Control, No. 1	7	0
Control, No. 2	0	0

[1]Seed germination checked on the 14th day after chemical treatment.

[2]Seedlings were checked for anomalous growth patterns until they had more than 22 mature leaves.

It has been suggested that the basis of promotive activity by thioamide groupings appeared to be necessary to obtain seed germination (6). During this investigation, tests were conducted in which seeds were supplied simultaneously with a thiol-containing compound, mercaptoethanol, and an amide-containing compound, urea. The latter compound, an oxygen analog of thiourea, could possibly form an imido group in a similar way to that suggested for thiourea. Instead of tautomerism between the divalent sulfur and the amide, it would occur between the divalent oxygen and the amide. The failure of the dormant seeds to respond to combinations of urea and mercaptoethanol would seem to indicate that the stimulation of peach seed germination by thioamides depends on a specific spacial arrangement of the reactive chemical groups, or at least requires that such groups be present in the same molecule.

The effect of thiourea on the subsequent development of seedlings from unchilled seeds was of considerable interest. A high percentage of seedlings grown from seeds treated with this chemical developed anomalous leaf patterns. It has been previously suggested that the retarding effect of high concentrations of thiourea on the growth of 'Lovell' peach seedlings from non-afterripened seeds might result from an inhibition of cell division (6). Since growth anomalies are prevalent in seedlings grown from non-afterripened seeds and would appear to be the result of an interference with cell division, it is possible that thiourea just enhances this phenomenon.

Since it has been shown that gibberellic acid can decrease, to some extent, the occurrence of leaf anomalies on peach seedlings and can stimulate stem elongation (5), it is possible that seedlings developing from seeds forced to germinate by gibberellic acid were also supplied enough of the chemical to decrease the occurrence of growth anomalies.

Summary

Gibberellic acid, 2-mercaptoethanol, 2-mercaptoethylamine, thiourea, and urea were tested for their influence on the germination of seeds of *Prunus persica* cv. 'Okinawa' and on the subsequent development of the seedlings. Thiourea was found to be a very effective promoter of peach seed germination, but seedlings from seeds treated with this chemical developed anomalous leaves. Gibberellic acid also stimulated seed germination, but to a lesser extent than thiourea

under the conditions of these tests. However, the seedlings produced from seeds treated with gibberellic acid did not develop the abnormal pattern of growth. Neither thiols nor amides, either alone or in various concentration combinations between the two chemicals, were effective in promoting 'Okinawa' seed germination. This would seem to support the suggestion made previously (6), that thioamides, e.g., thiourea and thioacetamide, owe their effectiveness as stimulators of peach seed germination to a specific molecular arrangement, possibly the formation of a thiolimido configuration.

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