PHYTOTOXICITY OF IMIDAZOLINE DERIVATIVES
AND RELATED COMPOUNDS

SEWARD E. ALLEN AND FOLKE SKOOG

(WITH FOUR FIGURES)

Received November 15, 1950

Introduction

In recent years many types of chemicals have been tested for activity in promoting or inhibiting growth of plants. The present paper reports on phytotoxic effects and on relationships between chemical structure and activity of imidazoline derivatives and related compounds, oxazolines, etc., some of which are extremely toxic to higher plants.

Certain imidazoline derivatives and related substances such as benzedrine and adrenaline have long been known to possess heart-stimulating activity. HARTMAN and ISLER (2) reported that 2-benzyl imidazoline was highly effective as a heart stimulant, but that its activity was eliminated by replacing the benzyl group with aliphatic chains with the exception of the chains containing six to eight carbon atoms. More recently BELLMONT and MEIER (1) showed that certain imidazolines were 10 to 100 times more effective than adrenaline.

As regards physiological effects of imidazolines on plants, these compounds were selected for testing in 1947 solely on the basis of their structural relationship to a substituted benzyl imidazolidinethione, which we had found to possess certain growth-regulatory properties. However, WELLMAN and MCCALLAN (11) and THURSTON et al. (7) have studied the fungicidal action of substituted 2-heptadecyl imidazolines and noted that toxicity to plants increased as the chain length in 2-position was shortened to C_{13} and C_{11}. Furthermore, toxicity was increased by lengthening the chain in 1-position to C_{6}, whereas the toxicity was markedly decreased by either complete elimination of this chain or by the introduction of a polar group onto the molecule.

Material and methods

The chemicals used are listed in part in table I and will be further specified in connection with the results. All compounds were tested first for their effects on the growth of germinating seeds, by a technique similar to that of SWANSON (6). Seeds of wheat (*Triticum vulgare* Vill., variety Henry) and radish (*Raphanus sativus* L., variety Scarlet Globe) were germinated on filter paper in Petri plates moistened with 5 cc. of a water solution or suspension of the compound under observation. Ten seeds were used in each plate and all treatments were duplicated throughout a six-step concentration range from 0 to 500 p.p.m. After four days of growth in an air-conditioned

---

1 Present address: Texas Research Foundation, Renner, Texas.
dark room maintained at 24° C and about 88% relative humidity, the seedlings were removed and the shoots and primary roots were measured. Statistical analysis of several typical results indicated that variations up to 20% might occur in a given experiment and all interpretations of results are made accordingly.

Compounds which proved to be toxic in the above tests, or were of interest because of their similarity to more active compounds, were further tested for their effects on the growth of larger plants in the greenhouse. These tests included spray applications to the aerial parts of the plants and addition of solutions to the soil in which the plants were grown. All spray tests were made with sufficient volumes to completely wet the foliage and all soil tests were made with enough solution to saturate the soil in the pots.

1-isopropyl-2-nonyl-4,4-dimethyl-2-imidazoline, which was highly toxic in all these tests, was used in respiration experiments. The conventional Warburg technique was employed. Discs of fresh leaf tissue were suspended in phosphate buffer of pH 7.0 and imidazoline was added from the side arm.

Finally, the two most toxic imidazolines were utilized in the field for control of weeds in corn and soybean plots. Further details of experimental procedures will be given in connection with the results.

**Results**

**IMIDAZOLINES.**—Compounds tested were 1-isopropyl-4,4-dimethyl-2-imidazolines of the following type:

![Chemical structure](https://i.imgur.com/3Q5z5.png)

The R-group in 2-position represents alkyl groups 1 to 17 carbon atoms long. The results obtained with ten of these compounds, all used at a concentration of 100 p.p.m., in the germination test, are summarized in figure 1. It may be seen that compounds with R-groups shorter than C7 were only slightly toxic. Increases in the length of the R-group to C7, C9, and C11 greatly enhanced toxicity, while a further increase to C17 caused a marked reduction in toxicity. However, unsaturation of C17 chains restored toxicity to a level comparable with that of the most active members of the series. With higher than 100 p.p.m. concentrations of compounds whose R-groups were C7 or longer seedling inhibition was complete. Moreover, compounds with R-groups of five or less C atoms produced lower than 50% inhibition, even at 500 p.p.m. The rather specific chain length requirement for high toxicity is strikingly illustrated by the 10-fold increase in toxicity caused by the substitution of the C7 for the C5 alkyl group.

The same 10 compounds were used in spray experiments with six-inch tomato plants. Six days after treatment, all plants were harvested and the
Fig. 1. Seedling response to 10 1-isopropyl-4,4-dimethyl-2-imidazolines differing only in the nature of the R-group at 2-position. (=) double bond in R-group at 8-position. (==) double bonds in R-group at 8,11-positions.

The differences in fresh weight of control and sprayed plants were used as a measure of toxicity. The results obtained with 0.06%' spray concentrations, summarized in figure 2, show effects and relationships between structure and activity similar to those obtained in the germination tests. Compounds with alkyl groups of seven or more C atoms either killed or did extreme damage in concentrations as low as 0.06%, while compounds with R-groups of five or less C atoms, even in 1% concentration, produced no measurable toxic effects.

Plants treated with the most active compounds lost turgidity within one hour and large areas of nearly colorless tissue developed on their leaves. The plants dried so quickly that death ensued within 48 hours. Plants treated with non-lethal concentrations usually recovered slowly from severe tissue dehydration which was accompanied by moderate to severe leaf abscission. Only very slight formative effects were observed.

Oxazolines.—To obtain more information on the relationship between chemical structure and toxicity, several oxazolines of the following type were compared in the germination test:

\[
\begin{align*}
\text{CH}_2 & \quad \text{CH}_2\text{OH} \\
\text{O} & \quad \text{C} \\
\text{CH}_3 & \quad \text{CH}_3 \\
\text{C} & \quad \text{N} \\
\text{R} & 
\end{align*}
\]

The R-group in 2-position represents alkyl groups of from five to 17 carbon atoms.
FIG. 2. Fresh weights of tomato plants harvested six days after spraying with 10 1-isopropyl-4,4-dimethyl-2-imidazolines differing only in the nature of the R-group at 2-position. (=) double bond in R-group at 8-position. (==) double bonds in R-group at 8,11-positions.

The results obtained at 100 p.p.m. concentration are summarized in figure 3. All oxazolines tested were much less toxic than imidazolines with corresponding R-groups and the peak was apparently reached with an R-group two carbons shorter than in the imidazoline series.

All oxazolines of this series contained a hydroxyl group in the 4-position which might account for the low toxicity of these compounds. However,
2-undecyl-4,4-dimethyl-2-oxazoline was also tested and found to be somewhat more toxic than oxazolines of the above series, but much less toxic than comparable imidazolines. It was concluded therefore that the nature of the ring also contributed to the toxic properties of the imidazoline derivatives. None of the oxazolines was considered sufficiently toxic to warrant greenhouse tests.

Other compounds.—Several compounds structurally related to imidazolines were compared in the germination test. The results obtained at 100 p.p.m. concentration are summarized in table I. It should be noted that, in every case, introduction of one or more polar groups greatly reduced toxicity of otherwise comparable molecules. From a comparison of these results with those obtained with imidazoline and oxazoline derivatives, it was deduced that the toxicity of the most active imidazolines was dependent upon the following structures: (a) a ring nucleus containing nitrogen and carbon atoms linked by one or more double bonds; (b) the lack of polar substituents; (c) a side chain of sufficient length to impart surface-active properties to the molecule.

Respiration experiments.—The high toxicity and rapid action of certain imidazolines suggested that these substances have marked effects on the metabolism of plants, which might be detected by measurements of respiration. Discs of leaf tissue from young plants of wild mustard (Brassica kaber Wheeler), redroot pigweed (Amaranthus retroflexus L.) and lambs-quarter (Chenopodium album L.) were floated in phosphate buffer (pH 7.0) in Warburg vessels maintained at 25°C. Oxygen consumption was measured for one hour before and five hours after the addition of 1-isopropyl-2-nonyl-4,4-dimethyl-2-imidazoline from the side arm. A five-step
TABLE I

INHIBITION OF SEEDLING ROOTS PRODUCED BY TREATMENT WITH A HIGHLY TOXIC IMIDAZOLINE AND CLOSELY RELATED COMPOUNDS.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>% inhibition of root growth (100 p.p.m. conc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-isopropyl-2-undecyl-4,4-dimethyl-2-imidazoline</td>
<td><img src="image" alt="Structure" /></td>
<td>95 98</td>
</tr>
<tr>
<td>1-(2-ethyl hexyl)-2-undecyl-1,4,5,6-tetrahydropyrimidine</td>
<td><img src="image" alt="Structure" /></td>
<td>63 69</td>
</tr>
</tbody>
</table>
### TABLE I (continued)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>% inhibition of root growth (100 p.p.m. conc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-amino-1,3-bis(1,3-dimethyl butyl)-5-methyl hexahydropyrimidine</td>
<td><img src="image1.png" alt="Structure 1" /></td>
<td>51 56</td>
</tr>
<tr>
<td>5-amino-1,3-bis(1,1-dimethyl-2-hydroxyethyl)-5-methyl hexahydropyrimidine</td>
<td><img src="image2.png" alt="Structure 2" /></td>
<td>0 4</td>
</tr>
<tr>
<td>Compound</td>
<td>Structure</td>
<td>% inhibition of root growth (100 p.p.m. conc.)</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>-----------------------------------------------</td>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>3-benzyl-5,5-dimethyl-2-imidazolidinethione</td>
<td><img src="image1" alt="Structure" /></td>
<td>Wheat</td>
</tr>
<tr>
<td>3-benzyl-5,5-bis(hydroxymethyl)-2-imidazolidinethione</td>
<td><img src="image2" alt="Structure" /></td>
<td>6</td>
</tr>
<tr>
<td>Compound</td>
<td>Structure</td>
<td>% inhibition of root growth (100 p.p.m. conc.)</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>-------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wheat</td>
</tr>
<tr>
<td>2-undecyl-4,4-dimethyl-2-oxazoline</td>
<td><img src="image" alt="Structure" /></td>
<td>33</td>
</tr>
<tr>
<td>2-undecyl-4-methyl-4-hydroxy-methyl-2-oxazoline</td>
<td><img src="image" alt="Structure" /></td>
<td>0</td>
</tr>
</tbody>
</table>
concentration range from 0 to 500 p.p.m. of imidazoline was used and all treatments were in duplicate. The results obtained at 250 p.p.m. (c. $10^{-3} M$) are summarized in figure 4.

Mustard tissue proved to be extremely sensitive to imidazoline, as respiration was inhibited over 90% after five hours exposure to concentrations as low as 60 p.p.m. Redroot pigweed tissue was considerably less sensitive and its oxygen consumption actually increased for the first hour after exposure to imidazoline. Lambsquarter was apparently unaffected at least during the 5-hour period of measurements.

Soil treatments.—Uniform six-inch tomato plants were treated with the three most toxic imidazolines at rates of 25, 50, 100, and 200 mg. per pot. All chemicals were dissolved in sufficient water to saturate the soil in the pots. The plants were harvested 16 days after treatment. No measurable growth differences or visible symptoms of toxicity were obtained. Previous data had shown that less than 2 mg. of imidazoline was sufficient to kill comparable tomato plants when the toxic material was sprayed on the foliage, and its addition to root systems in nutrient solutions was also toxic. It was concluded therefore that the toxic effects of imidazolines were enormously reduced as a result of contact with the soil.

Translocation of imidazolines within the plant.—Uniform tomato plants were fitted with wax paper shields to facilitate spraying particular parts without wetting other parts. When plants were sprayed on the upper half only, all treated parts were killed in a few days. With large plants, secondary buds on the untreated parts developed rapidly, as might be expected from decapitated plants. Small plants responded similarly, except that new organs were slightly malformed. This would indicate that a small amount of toxic material had been translocated downward.

With plants treated on the lower half only, all treated leaves soon abscised and the stems became discolored and dried. However, the toxic material did not stop the functioning of conductive tissues, as the untreated upper parts continued to grow in all cases. It was therefore concluded that only a very small part of the toxic material was translocated within the tissues.

Species relationships.—The laboratory and greenhouse tests indicated that certain imidazolines might be sufficiently toxic to be of practical use as herbicides. As a survey of this possibility, preparatory to field experiments, nine species of crops and weeds were grown in the greenhouse, and young plants, four to six inches tall, were sprayed with the most toxic compounds in 0.03 to 1% concentrations. In nearly all cases, the compounds with C9 and C11 chains were most toxic. The relative sensitivities of the nine species tested may be summarized as follows: (1) Resistant (minimum lethal spray concentration 0.25%): wheat (Triticum vulgare Vill., variety Henry), corn (Zea mays L., variety Wisconsin #595), lambsquarter (Chenopodium album L.); (2) Moderately Resistant (minimum lethal spray concentration 0.10%): soybean (Soja max Piper, variety Manchu), pea (Pisum sativum L., variety Yukon), beet (Beta vulgaris L., variety Detroit Dark Red), red-
root pigweed (*Amaranthus retroflexus* L.); (3) Slightly Resistant (minimum lethal spray concentration 0.05%): tomato (*Lycopersicon esculentum* Mill., variety John Baer), wild mustard (*Brassica kaber* Wheeler).

With all species, the most obvious toxicity symptoms were rapid loss of turgidity and extreme desiccation of the tissues. In a few cases, moderate formative effects were observed on plants treated with non-lethal concentrations. Additional experiments with four of the above species showed that resistance increased with age or size of the plants.

**FIELD EXPERIMENTS.**—A small experiment was designed to test the use of 1-isopropyl-2-nonyl-4,4-dimethyl-2-imidazoline for weed control in corn. Quadruplicate 2 by 10 hill plots of Wisconsin #595 were sprayed at two stages of growth, immediately before or 10 days after emergence of the seedlings. Imidazoline was applied at 0.5, 1 and 2 lbs. per acre in 80 gallons of water (1.6 liters per plot).

The pre-emergence treatment was made 13 days after planting, which was the last possible day before emergence of the corn seedlings. Unfavorable weather had delayed corn emergence. Weeds, mostly *Amaranthus retroflexus*, were about one inch tall at the time of the treatment. Nine days later, weed counts on all plots showed 90% control of broadleaf weeds by the 2 lbs. per acre application. Corn and grasses were unaffected.

The post-emergence treatment of weeds and corn two to six inches tall was less successful, as moderate damage was done to the corn without satisfactory control of weeds. It was concluded, therefore, that imidazolines were best adapted for control of seedling weeds before emergence of the crop plants.

A similar experiment with 4 lbs. per acre of imidazoline applied in 40, 80, and 120 gallons of water suggested that the compounds were not adapted to low-gallonage spray techniques. In addition, five by 12 foot plots of Manchu soybeans were sprayed in quadruplicate with 2, 4, and 6 lbs. per acre of 1-isopropyl-2-undecyl-4,4-dimethyl-2-imidazoline. Unfortunately, very few weeds developed on the plots prior to treatment so that the results gave primarily a measure of toxicity to the beans. Pre-emergence treatments did not cause damage but post-emergence applications caused moderate injury at all concentrations. As in the experiment with corn, grasses were relatively unaffected. It was concluded that pre-emergence treatment of soybeans might give satisfactory results, but post-emergence applications should be made with caution.

**Discussion**

The experiments reported here should be considered only as a basis for further experimentation. However, with the information obtained, some suggestions can be made regarding the mode of action and possible uses of the compounds. The relationships between toxicity and chain length of the substituent groups in 2-position, as well as the effects of substituent polar groups, indicate that the toxicity of the imidazolines is associated with their surface-active properties. Veldstra (8, 9) and Veldstra and Booij (10)
have emphasized that surface activity is an important property contributing to the activity of plant growth substances. They point out that all active compounds contain both hydrophilic and lipophilic groups and are, therefore, active at water-lipoid interphases. They claim that the physiological activity, especially the toxicity of compounds which affect permeability, is dependent upon a delicate balance between hydrophilic and lipophilic linkages, even if other more subtle properties of the molecules are responsible for their specific growth-regulatory functions within the cell.

The results obtained with the present group of toxic compounds are in agreement at least in part with Veldstra's (8) concept. The differences in chain length requirements for imidazolines and oxazolines may be interpreted by Veldstra's (9) theory of lipophilic/hydrophilic balance. The imidazoline nucleus is more hydrophilic than the oxazoline nucleus and a slightly longer R-group is required to give the lipophilic overbalance necessary for maximum toxicity. The decreases in toxicity produced by adding polar groups to oxazolines, imidazolidinethiones and hexahydropyrimidines may also be attributed to disruption of the lipophilic/hydrophilic balance.

The above concepts would also explain the decrease in toxicity produced by lengthening the R-group to C17. Such long chain compounds are so surface active that they tend to form micelles which greatly reduce the number of single molecules available for a specific reaction. Unsaturation of the long chain reduces the tendency toward micelle formation with resultant increase in toxicity.

It must be emphasized, however, that toxicity cannot be attributed solely to surface activity. Non-ionic detergents are highly surface active but are not toxic (Prill, Barton, and Stolt, 3). This suggests that the imidazoline nucleus itself is toxic, but may be prevented from getting into position to react due to its hydrophilic nature. With the addition of a surface-active R-group, lipophilicity is increased so that the molecule may be absorbed by the membrane and may either react with the membrane or penetrate it to exert a toxic effect in the cytoplasm. Whether or not the effect is mainly on the surface membrane or inside the cell, the toxic imidazolines drastically affect the water-holding capacity of the tissues. When the foliage is sprayed the air spaces between the cells become filled with liquid and very rapid loss of turgor and desiccation of the affected tissue ensue.

According to Putnam (4) detergents inactivate a variety of proteins and enzyme systems; also cationic detergents, such as imidazolines, react quantitatively with proteins. This property would be expected to account at least in part for the toxicity of imidazolines and may be responsible for the effect on respiration. With sensitive tissue, such as wild mustard, the minimum concentration of 1-isopropyl-2-nonyl-4,4-dimethyl-2-imidazoline required for nearly complete inhibition of respiration was considerably less than the required cyanide concentration. The actions of the two compounds, however, are hardly comparable as the effect of the imidazoline is irreversible and probably unspecific.

In view of the above considerations the toxic action of imidazolines may
be summarized tentatively as follows: (1) Surface-active imidazolines are adsorbed on the cell and may prevent its normal function in regulating passage of materials into and out from the cell; (2) Portions of the adsorbed compounds may enter the cytoplasm to react with protein and may thus cause irreversible inhibition of respiratory enzymes.

Another interesting point in the greenhouse and field experiments was the complete inactivation of the imidazolines by soil contact. As the compounds are strong cations, they are probably tightly attached to the negative surfaces of soil colloids, so that effective physical binding takes place before they can reach the plant roots. Similar binding of the compounds onto the negatively charged cell surfaces, together with their rapid toxic action, may explain in part their failure to be translocated in the plant. The apparent lack of translocation both in the soil and in the plant restricts the action of imidazolines to contact effects. Although this limits their general use as herbicides, it may be an asset in pre-emergence treatments of sensitive crops which are seriously damaged by after-effects of herbicides now in use.

**Summary**

Certain members of a series of 10 1-isopropyl-4,4-dimethyl-2-imidazolines with alkyl groups substituted in the 2-position were found to be toxic to seedlings and larger plants.

Their toxicity is a function of the length and degree of unsaturation of the alkyl group in the 2-position. Compounds with alkyl groups of five or less carbons were only slightly toxic; those with seven, nine, and 11 were extremely toxic; and those with 17 carbon atoms in a saturated chain were only moderately toxic. The presence of one or more double bonds near to the middle of the C\textsubscript{17} chain increased the toxicity of the latter compounds.

A comparable series of oxazolines was much less toxic, but showed a similar relationship between chain length and toxicity.

The substitution of one or more OH groups for H atoms in the methyl groups in the 4-position markedly reduced the toxicity of the compounds.

Related compounds, such as hexahydroimidazolines and imidazolidine-thiones, also possessed toxic properties which were largely removed by the incorporation of OH groups into the molecules.

The most toxic imidazoline derivatives applied as sprays to young tomato plants were lethal in concentrations of 0.05\%, whereas the least toxic ones produced no visible effects in concentrations of 1\%.

The toxic imidazolines are effective respiratory inhibitors, but are poorly translocated through plant tissues, and are inactivated by contact with soil.

The toxic action of the imidazolines may be due in part to their lipophilic properties.

In greenhouse tests with nine species: corn, wheat, and lambsquarter were found to be relatively resistant; soybeans, table beets, peas, and red-root pigweed were moderately sensitive; and tomatoes and wild mustard were readily killed by low concentration spray applications.
Limited field tests suggest that imidazoline derivatives may be of practical use for contact killing of plants, particularly of seedling weeds before the emergence of crop plants.

Acknowledgment is made to the Commercial Solvents Corporation for financial support and for preparation of chemicals; especially to T. S. Carswell and Robert Harker for their interest and helpful advice in these investigations. Facilities and assistance with field plot tests were kindly provided through Professor K. P. Buchholz, Department of Agronomy.

**LITERATURE CITED**