PRODUCTION OF YELLOW STRAPLEAF OF CHRYSANTHEMUM & SIMILAR DISORDERS BY AMINO ACID TREATMENT [1,2]

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An apparent physiological disease, Yellow Strapleaf (YSL) of *Chrysanthemum morifolium* Ram., was described by Jackson and Woltz (1) in 1959. The disease has been observed in Florida plantings during the last 6 years and has been noted in Massachusetts [3]. Symptoms include chlorosis of new growth, green netting of leaves, and narrow strap-shaped leaves that hook at the distal ends (fig 1A). The condition may persist for 2 to 8 weeks causing mild to severe stunting of plants and has been found in 20 commercial chrysanthemum varieties. YSL has not been corrected by the application of inorganic nutrient elements or linked directly with any fungal, bacterial, or viral pathogen. Chemical analyses of plant and soil have not indicated any significant differences in content of inorganic nutrients. Higher soil pH was usually associated with the disorder.

Woltz and Jackson (6) presented results of experiments that linked frenching of tobacco (*Nicotiana tabacum* L.) and YSL of chrysanthemum as being apparently caused by similar soil conditions and resulting in similar syndromes. Both disorders are favored by relatively high soil pH, periods of high soil moisture levels, and high soil temperatures. The symptoms displayed with the diseases (1, 5) have three common features: A: green netting or reticular chlorosis of leaves, B: narrow, strap-shaped leaves, and C: growth retardation. Tobacco and chrysanthemum plants growing together in containers of soil that had produced YSL in the field developed symptoms of the respective diseases of frenching and YSL. Following the information set forth by Steinberg (5) that isoleucine and certain other amino acids were effective in producing frenching, DL-isoleucine with DL-alloisoleucine was applied to the root zone of chrysanthemum plants. This amino acid treatment uniformly and quickly produced YSL symptoms with plants growing in solution culture, steamed soil, methyl bromide-treated soil, untreated soil, quartz sand and expanded volcanic glass (perlite). Six chrysanthemum varieties of varying degrees of susceptibility to YSL were treated with isoleucine in this experiment. YSL symptoms developed in all varieties with severity approximately in the order of observed field susceptibility.

This report presents data on the effects of certain specific amino acids on the growth of chrysanthemums.

METHODS

Rooted Iceberg variety chrysanthemum cuttings were planted March 11, 1960, in 4-inch plastic flower pots containing methyl bromide-treated sandy soil. One cutting in each of three replicate pots represented a treatment. Plants were fertilized weekly with a solution containing essential nutrient elements.

Amino acid and ammonium nitrate solutions of standard volume were applied to the soil surface around the base of each plant. Amounts applied, expressed in millimoles (mmoles) per plant, were the following: DL-alloisoleucine, 0.0125, 0.05, 0.2, and 0.8; D-alloisoleucine, 0.0125, 0.05, 0.2, and 0.8; L-isoleucine, 0.8 and 3.2; D-isoleucine, 0.8; L-leucine, 0.8, 3.2, and 6.4; D-leucine, 0.8; L-methionine, 0.8 and 3.2; D-methionine 0.8 and 3.2 mmoles; and controls consisting of varied amounts of ammonium nitrate to equal the amount of nitrogen supplied by each amino acid treatment. The L-isoleucine used was an allo-free grade. Other amino acid isomers were of the highest grade available from reputable biochemical suppliers.

The selection of dosage levels of the amino acid isomers was based on the results of preliminary experiments showing the relative effectiveness of the isomers as well as the available supply of the various isomers.

Half of the amount of amino acids intended for each plant was applied March 29 immediately after removal of the terminal growing points. The root systems were well established at this time. Three days later the remaining quantities of amino acids were applied. Heights of the uppermost new shoots were measured April 6, 14, and May 30, since growth retardation was considered the most significant, measurable index of YSL or other effects of amino acids. The plants were observed daily to note changes in leaf shape and the development of chlorotic patterns.

RESULTS

Mean heights of upper new shoots are given in figure 2. The heights of the new shoots at each of the dates are expressed on a relative basis, setting the

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[2] Florida Agricultural Experiment Station Journal Series no. 1113.

[3] Personal communication from E. C. Gasiorkiewicz, Sept. 30, 1959.

control means as 100. The same shoot on each plant was measured on each of the three dates. Growth increments were not used in the calculations. A positive slope between points indicates a tendency for recovery from the growth inhibiting effects of the amino acids. Negative or no slope indicates a failure to recover. The growth rate of plants recovering from the inhibitory effects of amino acids in some cases exceeded the growth rate of the control plants. For height comparisons at any given date of measurement, the LSD at the 5 % level is shown as 100 less the asterisk value. Any value below the asterisk differs significantly from the control.

In figure 2A the growth data for the four D-alloisoleucine levels are averaged and these mean values plotted since none of the individual levels gave growth values significantly different from the control and since there was no visible trend in their effect. Also plotted in figure 2A are the growth values for the individual levels of DL-alloisoleucine. Plants receiving 0.0125 to 0.8 mmoles of D-alloisoleucine exhibited no visible symptoms, whereas all treatments with DL-alloisoleucine produced YSL symptoms. Plants receiving the lowest amount of DL-alloisoleucine recovered from significant growth repression by the second measurement. Plants receiving the intermediate

FIG. 1. A: The chrysanthemum plant on the right is deformed and stunted by yellow strapleaf; the plant on the left, same age, is normal. Both plants are field-grown Iceberg variety. B: The plant on the left received 0.8 mmoles of DL-alloisoleucine producing yellow strapleaf symptoms. Center plant is a control. The plant on the right received 0.8 mmoles of D-methionine producing symptoms of methionine toxicity (methionosis). Plants were photographed 5 weeks after treatment.

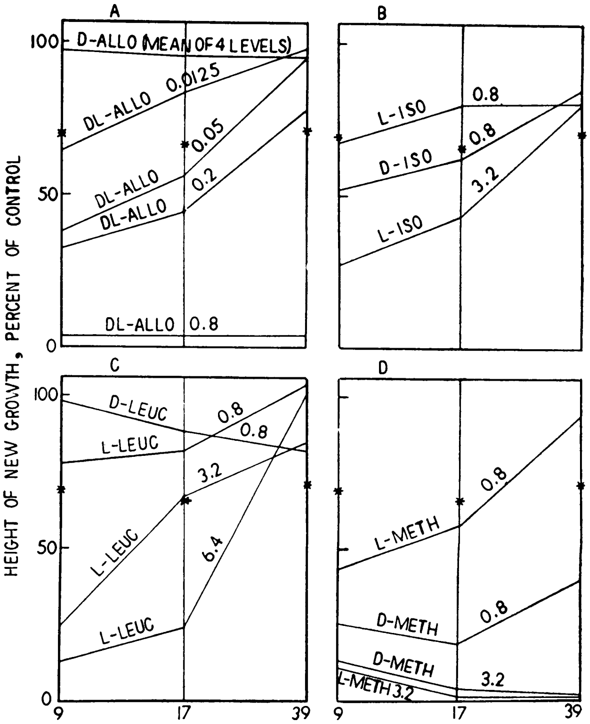

FIG. 2. Elongation of new shoots of chrysanthemum plants receiving indicated amounts in mmoles of isomers of A: alloisoleucine (Allo), B: isoleucine (Iso), C: leucine (Leuc), and D: methionine (Meth) applied to the root zone. Points below asterisks (LSD, 5 % level) differ significantly from controls for a given date of measurement.

amounts of 0.05 and 0.2 mmoles had recovered from significant repression by the third measurement. The greatest amount applied (0.8 mmoles) prevented any relative growth recovery (fig 1B).

Both D- and L-isoleucine produced YSL symptoms. The D isomer at the 0.8 mmoles level repressed growth both at the first and second dates (fig 2B) while the L isomer at the same level was not effective beyond the first date. L-isoleucine at 3.2 mmoles per plant repressed growth at the first and second dates of measurement: by the third date, however, the relative growth measurements indicated no significant difference.

D-leucine did not produce YSL symptoms at 0.8 mmoles while L-leucine at the same level produced slight, transient symptoms. Neither D- nor L-leucine significantly suppressed growth at the 0.8 mmoles level (fig 2C), although the L isomer had some effect in this direction. L-leucine at the 3.2 mmoles level repressed growth only at the first measurement. At the 6.4 mmoles level, growth was repressed at the first and second dates; by the third date, however, there was no significant difference.

D- and L-methionine both caused new leaves to become elongated in shape with entire margins and prominent midribs. Leaves developing later were round in shape. The plant with methionine toxicity in figure 1B had not developed final symptoms at the time of the photograph. The symptoms differ obviously, however, from those of YSL.

L-methionine at the 0.8 mmoles level (fig 2D) repressed growth at the first and second dates. The D-isomer at the same level was persistent in its effect through three dates. The difference in height between plants receiving D- and L- methionine at the time of the third measurement was quite pronounced indicating the L isomer was decreasing more rapidly in effectiveness with the passage of time. The results at the 3.2 mmoles level for both isomers were quite similar and the effect in growth suppression continued very strong at the third measurement date.

Undimensional paper chromatograms of leaves naturally affected with YSL showed at least 100 % greater total free amino acid content than normal leaves. Soils extracted with water and various reagents were found to contain free amino acids of leucine and isoleucine Rf values as well as others.

FOLIAR UPTAKE. Single plants of Iceberg variety chrysanthemum growing in methyl bromide-treated sandy soil in 4-inch plastic flower pots were used to determine whether DL-alloisoleucine taken up through the foliage would produce YSL symptoms. Pots were inclined and a leaf on one of the three shoots of each plant was immersed for 72 hours in A: 100 ml of deionized water (control) and B: 100 ml of 0.03 M DL-alloisoleucine. The controls exhibited no abnormalities while the younger leaves on the shoots above the treated leaves developed a green netting with chlorotic interveinal areas. This condition is characteristic of the first stages of YSL produced either naturally in the field or by the administration of isomers of isoleucine around the roots of the plant. Spraying the foliage of chrysanthemum plants with 0.075 M DL-alloisoleucine had no effect. By decapitating a shoot and retaining a 0.2 M solution of DL-alloisoleucine around the cut portion of the stem for 5 days it was possible to produce the same early YSL symptoms described above.

EXPERIMENTS WITH OTHER PLANTS. The possibility that naturally occurring disorders similar to YSL might be caused by amino acids in the soil was investigated. An assortment of genera representing 11 plant families was planted in containers of methyl bromide-treated soil in the greenhouse. Planting dates were selected so that the plants would be about the same size, 6 to 12 centimeters high, at the time of treatment. Plants were spaced about one per 60 square centimeters of area. Treatments, replicated three times were: A: DL-isoleucine containing unspecified amounts of DL-alloisoleucine, 1.2 mmoles/100 sq cm; B: L-leucine 1.2 and 4.8 mmoles per 100 square centimeters; C: DL-methionine, 0.6 mmoles/100 sq cm; and D: ammonium nitrate (control), 0.6 mmoles/100 sq cm.

The effects produced in all replications are described in table I, except for L-leucine which had no apparent effect. The symptoms produced by isoleucine are somewhat similar for all plants tested. Affected plants exhibited some or all of the general symptoms that follow: A: green netting of leaves, B: narrowing of leaf or leaflet, and C: abnormal leaf pattern in regard to vein and marginal characteristics (veins frequently were more prominent & leaf margins lost their lobate characteristics). An exception was *Zea mays* (corn) which had an entirely different syndrome as described in table I. Corn was the only grass tested. Grasses as a group may have an entirely different response.

Leaf symptoms resulting from isoleucine treatment of tomato plants were generally similar to the symptoms of tobacco mosaic virus disease of tomatoes. Squash and cucumber treated with isoleucine had leaf symptoms similar to the cucumber mosaic virus disease of these two plants.

DISCUSSION

Data of Putnam and Schmidt (4) together with preliminary, unpublished results of the present authors indicate that naturally-occurring free amino acids are available to plant roots under many soil conditions. It is well established in the literature (5) that plants are capable of taking up amino acids by way of their roots. These considerations coupled with the data in this report on the effect of isomers of leucine, isoleucine, and methionine lead to the suggestion that naturally occurring amino acids may act as growth inhibitors and constitute potential causal agents of abnormal plant growth in the field. YSL of chrysanthemum and frenching of tobacco are like-

TABLE I

SYMPTOMS PRODUCED BY APPLYING DL-ISOLEUCINE PLUS DL-ALLOISOLEUCINE & DL-METHIONINE TO ROOT ZONE OF INDICATED PLANTS

BINOMIAL	COMMON NAME	ISOLEUCINE	METHIONINE
Ambrosia trifida L.	Ragweed	Green netting of leaves	...
Antirrhinum majus L.	Snapdragon	None	None
Beta vulgaris L.	Beet	None	None
Bidens pilosa L.	Bur marigold	Green netting of leaves	...
Brassica oleracea v. gongylodes L.	Kohlrabi	None	...
Callistephus chinensis (L.) Nees	Aster	None	None
Capsicum frutescens L.	Pepper	Green netting of leaves	None
Cucumis sativus L.	Cucumber	Leaves small, chlorotic, very serrate, with prominent veins	...
Cucurbita pepo L.	Squash	Leaves narrow, chlorotic, sharp lobed, with prominent veins	...
Dianthus armeria L.	Grasspink	Green netting of leaves, stunting	None
Lathyrus odoratus L.	Sweet pea	None	None
Limonium sinuatum (L.) Mill.	Statice	None	None
Lycopersicon esculentum Mill.	Tomato	Green netting of leaves, with mottling & puckering; narrow, slightly twisted leaflets	None
Nicotiana tabacum L.	Tobacco	Green netting of leaves, stunted plants	...
Petunia hybrida Vilm.	Petunia	Green netting of leaves	None
Phaseolus vulgaris L.	Bean	Green netting of leaves, narrow leaves, short plants	Narrow leaves, prominent sometimes translucent veins, epinasty of leaf tip, cupping of leaves, short plants
Pisum sativum L.	Pea	None	None
Salvia farinacea Benth.	Blue salvia	Green netting, narrow leaves	...
Tagetes erecta L.	Marigold	Narrow, strap-shaped yellow leaves; loss of marginal indentations	None
Tropaeolum majus L.	Nasturtium	Green netting of leaves	None
Zea mays L.	Corn	Watery, brownish breakdown about middle of leaves, suggestive of calcium deficiency symptoms	None Same as for isoleucine
Zinnia elegans Jacq.	Zinnia	Green netting of leaves; strap-shaped, narrow leaves	None

ly examples of such occurrences. Both represent plant diseases of serious economic consequences in the field. That many plants other than tobacco and chrysanthemum may be affected was demonstrated by the development of definite symptoms in 15 of 22 additional genera tested.

Transient cases of YSL of chrysanthemum were produced by treatment with the appropriate isomers of leucine and isoleucine at the lower levels employed. Transient YSL also occurs in the field. In both instances the same succession of symptom development is generally as follows: A: a green netting of the leaf blade; B: development of partially deformed (loss of part of the normal lobate characteristic) new leaves; and C: the disappearance of the green netting that was present in the older leaves with the concurrent development of normal new leaves.

The symptoms caused by D- and L- methionine are similar to YSL in the early stages; however, as plant growth continues there is no question that the symptoms are different. The name "methionosis" is suggested for the condition produced by excess D- and/or L- methionine in the chrysanthemum plant. Approximately six weeks after treatment with methionine the upper leaves of chrysanthemum plants assume the appearance of Bibb lettuce leaves. The leaves are elongated, have an entire margin, a prominent midrib, and are thin and soft. YSL leaves, in contrast, are very narrow and rather hard in texture, frequently having a hooked tip (fig 1).

The fact that D-isoleucine and D-methionine are more persistent in continuing to produce symptoms than the L isomers may be due to the fact that they are so-called unnatural isomers that are not synthesized into ordinary plant protein. Some organisms contain D amino acid oxidase (2) to transform D-isomers that are not useful and which may, in addition, be toxic; whether chrysanthemums are so supplied is not known. Since D-alloisoleucine was ineffective at all rates and DL-alloisoleucine was highly effective and persistent it seems likely that L-alloisoleucine is the effective portion of the mixture. The literature indicates that L-alloisoleucine has not been found in plant proteins. The high degree of effectiveness and persistence of L-alloisoleucine in causing YSL symptoms suggests that it is not readily incorporated into plant protein.

It is not possible from the data in this report to reach any definite conclusion as to the exact cause of YSL or to the mode of action of amino acids in producing the symptoms. Nevertheless, it is hypothesized from the information at hand that natural YSL may be caused by the growth-modifying effects of certain isomers of leucine and isoleucine taken up by chrysanthemum roots under specific soil conditions. The likelihood of such an hypothesis is strengthened by reports (2, 3) that D-isomers of amino acids including alloisoleucine occur in various natural antibiotic compounds. Obviously, further work is required to clarify the effect of amino acids upon plant growth under natural conditions.

Summary

Symptoms of the yellow strapleaf disease were produced by the application of DL-alloisoleucine, D- and L-isoleucine, and L-leucine individually to the soil surface around the base of chrysanthemum plants. D-alloisoleucine and D-leucine were found to be ineffective in producing these symptoms. D- and L- methionine both caused new leaves to become elongated in shape with entire margins and prominent midribs. Leaves developing later were round in shape. The name "methionosis" is suggested to describe this condition. The height of new growth occurring after the removal of the growing point was significantly reduced only by the isomers that were effective in producing abnormal growth.

The hypothesis is made that yellow strapleaf may be caused by the accumulation of excess amounts of the isomers of isoleucine and leucine in the plant tissue in a free state and that under certain soil conditions such amino acids may be taken up by the roots in amount sufficient to provide the excess.

Plants from 22 different genera of 11 plant families were subjected to treatment with isomers of isoleucine, leucine, and methionine. Isoleucine was most effective, producing symptoms in 15 of 22 genera treated. Leucine was not effective while methionine produced significant effects in two genera.

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