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INVESTIGATIONS ON PLANT CHEMOTHERAPY

II. INFLUENCE OF AMINO ACIDS ON THE RELATION PLANT–PATHOGEN[1] [2]

Met een samenvatting: Onderzoekingen op het gebied van de chemotherapie II. Invloed van aminozuren op de relatie plant-pathogeen

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INTRODUCTION

In investigations concerning plant chemotherapy, VAN RAALTE, KAARS SIJPESTEIJN, VAN DER KERK, OORT & PLUYGERS (1955) have shown that S-carboxymethyl-N,N-dimethyldithiocarbamate (in this article referred to as carboxymethyl-DDC) after intake by the root of cucumber plants increases the resistance of the above-ground parts against the fungus *Cladosporium cucumerinum*. Carboxymethyl-DDC is therefore a compound with systemic activity, having moreover the remarkable property that in vitro it can be hardly called a fungicide: it only exerts an influence within the plant. This protective influence might be explained in two ways, viz. by assuming that within the plant the compound is decomposed and produces the fungitoxic thio- or dithiocarbamate ion or by taking that the compound influences the metabolism of the plant and in that way would increase the resistance.

The latter possibility induced the present writer to study the influence of amino acids on the effect of carboxymethyl-DDC. For this purpose the plants were placed in solutions of amino acids ascertaining their effect with or without carboxymethyl-DDC. The experiments showed that several amino acids themselves greatly influence the severity of the disease, that is the relation plant-pathogen.

In some publications only mention is made of an influence of amino acids on the susceptibility of plants to pathogens. For instance ALTEN & ORTH (1941) found that in potatoes there is a correlation between resistance to *Phytophthora infestans* and the arginine content, such that a higher content of arginine occurs in more resistant varieties. KLEIN (1956) established that large dressings of ammonium nitrogen increased the glutamic acid and glutamine content in tobacco, which correlated with a higher susceptibility to some fungi. KUĆ et al. (1957), in actively growing shoots of apples, brought phenylalanine into the petioles and 24 hours later they inoculated the leaves with *Venturia inaequalis*. This appeared to decrease the infection.

After discussing the methods and material used we will first give a survey of the effect of amino acids and related compounds. The results obtained led to some preliminary investigations on the influence of the active amino acids on

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the pathogens in vitro. The few experiments on the influence of amino acids on the development of the host plant are considered in the discussion as well as the research of the fungicidal influence of sap obtained from plants treated with amino acids.

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MATERIAL AND METHODS

Most of the investigations were carried out with the cucumber as a test plant and *Cladosporium cucumerinum* ELL. et ARTL. as the pathogen. A number of experiments was repeated later with other plant-pathogen combinations in order to ascertain whether the effects found were of a general or of a more specific nature.

Cucumber (*Cucumis sativus*. L.).

Cucumber plants are very suitable as test plants since they are easy to grow and because the incubation period after inoculation with *Cladosporium cucumerinum* is short. The variety Lange gele Tros was used in the experiments. The seedlings were grown in partially sterilized sand, and, when they were seven to ten days old (the cotyledons have then developed fully but no leaves have been formed) they were placed with their roots in solutions of the compounds in water. After two days the roots were rinsed and placed in water; the plants were then sprayed with a conidial suspension of *Cladosporium cucumerinum*. After staying in a moist environment at 18° to 20°C for five to seven days the hypocotyls showed symptoms of the disease, but the cotyledons were seldom affected, so that only the hypocotyls were considered in estimating the disease index. The latter was graded in a scale from 0–6, in which 0 meant healthy and 6 very heavily diseased. The total disease index for every treatment was obtained by adding the products of every disease index and the number of plants having that disease index. The experiments were not carried out under constant conditions and the variation was considerable. In order to simplify the mutual comparison of the different experiments we have always put the total disease index of the control plants at 100 and expressed the severity of disease of the treated plants as percentage of the control. For example, if ten control plants all have a disease index of 6, and treatment with substance X results in four plants with the index 6, three plants having the index 3 and three having the index 1, then the severity of disease will be $\frac{36}{60} \times 100 = 60\,\%$. It will be clear that the estimation itself is not very accurate and differences of less than 10 % certainly cannot be considered real.

In these experiments only the preventive effect of the compounds was ascertained.

In a number of experiments older cucumber plants were used instead of seedlings. These were grown in soil and used when two or three leaves had developed. The way of treatment will be mentioned in the discussion of the separate experiments. Just as with the seedlings, the plants were treated before the inoculation, unless mentioned otherwise. The inoculation was carried out in the same way as in the seedlings, but it lasted six to ten days before the disease index could be assessed. Particularly the vines, buds and young leaves were affected. The symptoms of the whole plant were considered in the estimation. The grade for severity of disease was obtained by using a fourfold scale (healthy = 0, heavily diseased = 3). The computation of the severity of disease as percentage of the control was carried out as described above.

The results obtained with cucumbers were supplemented with experiments with other plants.

Broad bean (*Vicia Faba* L.)

Plants of the variety Con Amore with two fully developed leaves were placed with their roots in the solutions to be tested. After two days the solutions were replaced by water. The plants were sprayed with a suspension of conidia of *Botrytis fabae* SARD. and kept under moist conditions at 23 °C; 24 hours after inoculation the number of spots on an area of 18 × 18 mm^2 of one leaflet of each leaf was counted (FAWCETT, SPENCER & WAIN, 1955). A distinction was made between large and small spots. In the tables the average of the total number of spots per plant of the control has been put at 100, so that the severity of disease could again be expressed as percentage of the control.

Tomatoes (*Solanum Lycopersicum* L.)

Plants with four or five leaves of the variety Tuckqueen were inoculated with *Phytophthora infestans* (MONT) DE BARY by spraying with a suspension of zoospores. The disease index was assessed after four to five days in a moist environment at 18° to 20 °C. A fourfold scale was used in which healthy = 0, heavily diseased = 3. As in the preceding treatments the severity of disease was obtained by expressing the total disease index for any treatment as percen-ntage of that of the control. The solutions were applied before the inoculation.

The concentrations of the solutions used have in general been indicated in percentages. In some experiments they have been computed in mol. in order to enable a better mutual comparison. The pH of the solution was brought on 5.5 to 6 by adding potassium hydroxide or hydrochloric acid if necessary.

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Solutions of 0.50 and 0.25 % were applied and when these appeared to be phytotoxic, also solutions of 0.10 and 0.05 % were used. The phytotoxicity was judged only by sight according to the turgescence of the roots and the whole plant, according to the injury of the cotyledons etc. As from some experiments it had become clear that there are amino acids which only exert an influence in combination with a fungicide, all experiments were carried out both without and with addition of such a fungicide. For that purpose in particular sodium-dimethyldithiocarbamate (NaDDC) was used in a concentration of 100 or 50 ppm. From the results given in tabel 1 it appears that the amino acids can be roughly divided into three groups:

TABLE 1. Effect of amino acids on the infection of cucumber seedlings by *Cladosporium cucumerinum*.

Effect van aminozuren op de aantasting van komkommerkiemplanten door Cladosporium cucumerinum.

	Activity in seedling test *Werkzaamheid in komkommertoets*		Toxicity for cucumber* *Toxiciteit voor komkommers*
	without NaDDC *zonder* ,,	with NaDDC *met* ,,	
DL-serine	++	+++	±
D(+)-serine	++	+++	±
L(—)-serine	±	±	–
DL-threonine	++	+++	±
D(+)-threonine	++	+++	±
L(—)-threonine	±	±	–
DL-allothreonine	–	–	+
L(—)-threo-β-phenylserine	++	+++	±
DL-histidinemonohydrochloride	+	+	–
D(+)-histidinemonohydrochloride	+	+	±
L(—)histidinemonohydrochloride	–	–	–
glycine	–	++	–
DL-alanine	–	++	–
D(—)-alanine	–	+	±
L(+)-alanine	–	±	–
sarcosine	–	++	±
betaine	–	–	+
DL-β-alanine	–	±	+
DL-α, β-diaminopropionic acid	+	+	+
DL-phenylalanine	–	–	–
DL-dioxyphenylalanine	?	?	–
DL-α-aminobutyric acid	–	–	+
DL-β-aminobutyric acid	–	±	+
γ-aminobutyric acid	–	–	–
DL-α-aminoisobutyric acid	–	–	+
DL-β-aminoisobutyric acid	–	±	+
DL-norvaline	–	–	+
L(—)-valine	–	–	+
norleucine	–	–	–
D(+)-leucine	–	–	+
D(—)-isoleucine	–	–	–
DL-lysinedihydrochloride	–	–	+
DL-aspartic acid	±	±	–
L(—)-asparagine	–	–	–
DL-glutamic acid	–	–	–
L(+)-glutamine	–	–	–
L(—)-cysteinehydrochloride	–	–	++
L(—)-cystine	–	–	–
DL-methionine	±	±	–
DL-aethionine	–	–	+
L(+)-ornithinedihydrochloride	–	–	+
DL-citrulline	–	–	–
L(+)-arginine base	–	–	–
DL-proline	±	±	++
hydroxyproline	±	±	++
DL-tryptophane	–	–	–
glycylglycine	–	++	–
DL-alanylglycine	–	++	–
glycyl-DL-phenylalanine	–	–	–
diketopiperazine	–	–	–

* ± = toxic in concentrations of 0.50% and more; + = toxic in concentrations of 0.50–0.10%; ++ = toxic in concentrations of less than 0.10%.

± = toxisch in concentraties van 0,50% en hoger; + = toxisch in concentraties van 0,50–0,10%; ++ = toxisch in concentraties van minder dan 0,10%.

A. Amino acids which applied alone produce a marked increase in resistance against *Cladosporium cucumerinum* (serine-group and histidine).

B. Amino acids, which have no influence when applied alone, but which do increase the resistance in combination with NaDDC and other fungicidal compounds (glycine-group).

C. Amino acids which have no pronounced effect, whether applied separately or in combination with NaDDC.

A. Amino acids of the serine-group and histidine

Among the amino acids of group A are in the first place serine, threonine and threo-β-phenylserine, secondly histidine. The effect of the serine-group is given first, histidine being discussed afterwards.

The first mentioned compounds have the formule COOH.$CHNH_2$.CHOHR in which R may be substituted by H, CH_3 or a phenyl group. The influence of DL-serine is apparent from table 2. Diaminopropionic acid, in which the hydroxyl group of serine is substituted by an amino group was also investigated. The phytotoxic effect of this compound appeared to be too great to allow a conclusion. In most cases we used DL-serine and DL-threonine while we had at our disposal only the L-form of threo-β-phenylserine. The activity of the optical isomers was compared in a separate experiment. L-serine and L-threonine proved to have practically no influence on the resistance while the D-forms were very active (table 3). DL-allothreonine gave no protection in contrast to DL-threonine. KUĆ et al. (1957) also found that only D-phenylalanine protected apples against *Venturia inaequalis*.

TABLE 2. Effect of amino acids of group A. Cucumber seedling test.
Invloed van de aminozuren van groep A. Komkommerkiemplantentoets.

Concentration	Severity of disease in % *Mate van aantasting in %*		
	DL-serine	DL-threonine	DL-histidine
Control (water)	100	100	100
0.025 M	79	24	51
0.050 M	46	14	16
0.075 M	26	2	*

* Phytotoxic

TABLE 3. Effect of stereoisomers of serine. Cucumber seedling test.
Effect van de stereo-isomeren van serine. Komkommerkiemplantentoets.

Concentration	Severity of disease in % *Mate van aantasting in %*		
	DL-serine	D(+)serine	L(—)serine
Control (water)	100	100	100
0.20%	60	43	86
0.40%	31	12*	93
0.80%	24*	–	–

* Phytotoxic effect on roots.
wortels beschadigd.

Active concentration. The lower limit of the active concentration lay, under the conditions of the experiments, between 0.50 and 0,25 % or 0.050 and 0.025 M for DL-serine (tabel 2), while L-threo-β-phenylserine had a marked effect at a concentration of 0.10 % (± 0.0055 M). When a mixture of DL-serine and DL-threonine in various ratios was used, an additive effect could be observed. A cumulative or antagonistic effect was out of the question.

Importance of nitrogen supply. Amino acids taken up by the roots may in some cases serve as a source of nitrogen (ROBBINS, 1951). In order to ascertain whether the increased resistance which was observed could be due to a better supply of nitrogen, the protective influence of anorganic nitrogen compounds was investigated. For that purpose the plants were placed in a nutrient solution according to HOAGLAND, which contained calcium and potassium nitrate. This had no effect. Solutions of ammonium salts of which the nitrogen content could be compared with that of the serine solutions used, had no influence either.

Application to the cotyledons. From the experiments described above it is clear that parts of plants which themselves have not been in contact with the amino acids were protected against the fungus; in other words, serine or a derivative compound was transported upwards. In order to ascertain whether uptake by the cotyledons was also possible the following experiment was made: agar was added to a quantity of the solution which was otherwise used for 10 plants; the agar plate was cut into 20 cubes and on both sides of the cotyledons of 10 plants a cube was fixed. After two days the agar was removed and inoculation took place in the usual way. It is difficult to compare the intake by roots and by cotyledons, but in this way both concentration and total quantity of the substance supplied were the same in both cases. Table 4 shows in both cases a protective effect when 0.50 % or more concentrated solutions were used. Apart from intake into the cotyledons there is apparently also some transport from the cotyledons downwards.

TABLE 4. Effect of application of DL-serine on roots and on cotyledons. Cucumber seedling test.

Effect van DL-serine, toegediend aan de wortels of de cotylen. Komkommerkiemplantentoets.

Concentration	Severity of disease in % *Mate van aantasting in %*	
	Applied on roots *Aan de wortels toegediend*	Applied on cotyledons *Aan de cotylen toegediend*
Control (water)	100	100
DL-serine 0.10 %	100	85
,, 0.25 %	93	83
,, 0.50 %	65	52
,, 1.00 %	9	26

Combination with other compounds. As was stated in the Introduction this effect of serine was found during an investigation of the influence of amino acids on the activity of carboxymethyl-DDC in cucumbers. While in most experiments only a 0.50 % solution of DL-serine gave a marked effect, a similar influence appeared to be exerted by a 0.10 % solution to which 100 ppm carboxymethyl-DDC was added (table 5; fig. 1 and 2). However, carboxymethyl-DDC itself shows systemic activity (VAN RAALTE et al. 1955) so that from this it could not be concluded whether this is a cumulative or an additive effect. In order to gain a better insight in this question a number of compounds related to carboxymethyl-DDC which in the cucumber test gave negative or very slight positive results, were applied in combination with DL-serine (table 6). In this case always a suboptimal concentration of serine was given in order to set off better the increase in effectivity. In the left column of table 6 is indicated the effect of the compound tested by itself, in the right column the effect in combination with 0.25 % DL-serine.

TABLE 5. Effect of DL-serine, applied without and in combination with carboxymethyl-DDC. Cucumber seedling test.

Effect van DL-serine alleen en in combinatie met carboxymethyl-DDC toegediend. Komkommerkiemplantentoets.

Concentration	Severity of disease in % / *Mate van aantasting in %*	
	Without carboxymethyl-DDC / *Zonder* ,,	With Carboxymethyl-DDC / *Met* ,,
Control (water)	100	100
DL-serine 0.10 %	100	31
,, 0.25 %	96	26
,, 0.50 %	42	0
,, 1.00 %	0	0

TABLE 6. Activity of carbamates and derivates (100 ppm solution) as influenced by addition of DL-serine. Cucumber seedling test.

Invloed van DL-serine op de werkzaamheid van carbamaten en verwante verbindingen in de komkommerkiemplantentoets.

	Severity of disease in % / *Mate van aantasting in %*	
	Without serine / *Zonder* ,,	With DL-serine 0.25 / *Met* ,,
Control (water)	100	50
S-carboxymethyl-N,N-dimethyldithiocarbamate (Carboxymethyl-DDC)	95	68
Sodiumdimethyldithiocarbamate (NaDDC)	69	3
Tetramethylthiurammonosulfide (TMTM)	100	14
Tetramethylthiuramdisulfide (TMTD)	86	5
S-(2-carbamoylpropyl-2)-N,N-dimethyldithiocarbamate	100	156
S-(2-carboxypropyl-2)-N,N-dimethyldithiocarbamate.	95	86
N,N-dimethyldithiocarbaminylglycolic acid	100	99

Some of the compounds were barely soluble; in that case a suspension containing 10 mg per 100 ml (100 ppm) was used. The effect of carboxymethyl-DDC, which is very variable, was extremely slight in this experiment; furthermore only NaDDC gave some protection. However, NaDDC as well as TMTD and TMTM when added in combination with DL-serine, gave a surprisingly good result. In contrast tō the other four compounds, NaDDC, TMTD and TMTM are good fungicides. S-carbomethoxy-N,N-dimethyldithiocarbamate and bis (N,N-dimethyldithiocarbamyl) acetic acid, which have also fungicidal activity, caused also an increase in the effect of DL-serine. Application of NaDDC in combination with DL-threonine and L-threo-β-phenylserine gave similar results. Again the D-forms of serine and threonine were active, while the L-isomers had hardly any influence. The question was now whether it was a specific effect of amino acids with dithiocarbamates or whether a more general phenomenon was involved. Some fungicides of different chemical composition were used with DL-serine in the cucumber test. They were phygon (2,3-dichloro-1,4-naphtoquinone) captan (N-trichloromethylthio-tetrahydrophtalimide) nabam (disodiumethylene bisdithiocarbamate) and 8-hydroxyquinoline. The results were less evident than those given in table 6. Presumably the strong phytotoxicity of these compounds plays a part here; the experiments with nabam and hydroxyquinoline allowed of no conclusions for that reason, the others point to a stronger effect (table 7). Again, similar results were obtained whether the solutions were applied to the roots or to the cotyledons. In a few experiments the roots were cut off and the hypocotyls placed in the solutions. In that case also DL-serine gave some protection against *Cladosporium cucumerinum*, which could be increased by adding NaDDC to the medium. It was remarkable that in the presence of DL-serine in the medium the fungicides were phytotoxic in a lower concentration than when they were used alone.

TABLE 7. Result of addition of DL-serine on the activity of phygon and captan. Cucumber seedling test.
Effect van DL-serine op de werkzaamheid van phygon en captan in de komkommerkiemplantentoets.

	Severity of disease in % *Mate van aantasting in %*	
	Without DL-serine *Zonder* ,,	With DL-serine (0.25 %) *Met* ,,
Control (water)	100	58
Phygon 20 ppm	72	3
Phygon 50 ppm	72	*
Captan 10 ppm	90	25
Captan 25 ppm	100	20
Captan 50 ppm	100	*

* Phytotoxic.

Duration of the protection.. From the above experiments in which the inoculation was always carried out after the treatment with the various compounds, it is clear that there must be some after effect. This was investigated more closely in an experiment whereby plants were inoculated not directly after the treatment but after they had first stood in water for a few days. Such an experiment is presented in table 8. It appeared that even when the inoculation took place a week after the treatment no decrease of the protective effect could be established.

Curative effect. As was mentioned above we mostly applied the compounds to the plants before the inoculation took place. In some experiments the seedlings were first inoculated and only 24 hours later they were sprayed with solutions of DL-serine or DL-serine and NaDDC. This gave a marked protection: the plants treated with DL-serine (0.25 %) showed a disease index of 56 % of that of the control; when treated with DL-serine combined with NaDDC the disease index amounted to 46 % of that of the control; spraying after 48 hours or longer had no effect. If the plants were not sprayed but placed with the roots

TABLE 8. Duration of protective effect. Inoculation 0, 3, 5 and 7 days after treatment Cucumber seedling test.

Duur van de beschermende werking. Inoculatie 0, 3, 5 en 7 dagen na de behandeling. Komkommerkiemplantentoets.

	Severity of disease in % *Mate van aantasting in %*				
	0	3	5	7	Days after treatment *Dagen na behandeling*
Control (water)	100	100	100	100	
NaDDC, 50 ppm	83	105	83	93	
DL-serine, 0.25 %	58	50	44	64	
DL-serine, 0.25 % NaDDC, 50 ppm	39	35	31	29	

in the solutions, a favourable effect was obtained only when this took place immediately after the inoculation. In this case the active substances must be transported first to the inoculated parts.

Cucumber plants. Plants with several leaves were either placed with the roots in solutions of DL-serine or the above-ground parts were sprayed with these solutions while the plants grew in soil. In both cases the severity of the disease decreased under the influence of the treatment. This is demonstrated in table 9: six plants were sprayed with various solutions, twice a day, during two days. Inoculation took place 18 hours after the last treatment.

TABLE 9. Effect of spraying the leaves of cucumber plants with DL-serine and NaDDC.

Effect van bespuitingen met DL-serine en NaDDC bij komkommerplanten.

	Severity of disease in % *Mate van aantasting in %*	
	Without NaDDC *Zonder* ,,	With NaDDC (100 ppm) *Met* ,,
Control (water)	100	93
DL-serine 0.25 %	66	47

When using more concentrated solutions of DL-serine, we obtained better results even without adding NaDDC. However, the treated plants were lagging behind in growth as compared with the control.

Also in this case a curative effect could be established. Plants were inoculated and kept under moist conditions for 24 hours. The next 24 hours they were sprayed with different solutions. Treatment with water and NaDDC did not influence the severity of the disease; treatment with solutions of DL-serine and NaDDC produced a pronounced decrease (fig. 3 and 4). DL-serine was again used in a suboptimal concentration, which accounts for its slight effect. If the treatment took place about 48 hours after inoculation, there was hardly any effect.

The influence of these amino acids on the susceptibility was also studied in some other plants.

Broad beans. The plants were placed with their roots in solutions of DL-serine for two days and then inoculated with *Botrytis fabae*. The number of spots on the leaves appeared to decrease as a result from a treatment with

0.25 % DL-serine, while relatively more small and fewer large spots were found (table 10). Enhancing the concentration to 0.5 % or more had little effect. A complete protection was obtained in none of the treatments.

TABLE 10. Protection of broad beans against *Botrytis fabae*, resulting from treatment with amino acids.

Bescherming van tuinbonen tegen Botrytis fabae *door behandeling met aminozuren.*

	Severity of disease in % *Mate van aantasting in %*
Control (water)	100
DL-serine 0.25 %	25
DL-threonine 0.25 %	37
Glycine 0.25 %	113

In this case a far smaller number of amino acids was tested than in the case of cucumber seedlings. DL-aspartic acid, DL-glutamic acid, L-asparagine, L-glutamine, DL-alanine, glycine, DL-methionine and DL-phenylalanine gave negative results as was also found in the cucumber seedling test. Adding carboxymethyl-DDC to the solutions did not influence the effect of DL-serine in contrast to the results obtained with cucumbers.

Tomato plants. In the same manner we investigated how far the resistance of tomato plants against *Phytophthora infestans* was affected by a previous treatment with amino acids. In some experiments the plants were placed with the roots in a solution. However, this presented practical difficulties. In other experiments the above-ground parts were sprayed with a solution of amino acids. The plants were more easily injured than in the case of cucumber or broad beans, so that solutions of 0.10 or 0.05 % DL-serine had to be used. Even then sometimes small necrotic spots appeared on the leaves. However, no growth inhibition was found.

TABLE 11. Effect of DL-serine and DL-serine and NaDDC on the infection of tomato plants with *Phytophthora infestans*. Solution sprayed on the leaves.

Effect van DL-serine en DL-serine met NaDDC op de aantasting van tomaten door Phytophthora infestans. *Bladeren bespoten met de oplossingen.*

	Severity of disease in % *Mate van aantasting in %*	
	Without NaDDC *Zonder* ,,	With NaDDC (100 ppm) *Met* ,,
Control (water)	100	118
DL-serine 0.05 %	70	46

The plants, of the experiment mentioned in table 11 were sprayed twice a day during two days, and inoculated one day after the last treatment. Both DL-serine and DL-serine with NaDDC appeared to decrease the susceptibility of the plants. It is remarkable that such an effect could be obtained at such a low concentration.

Carboxymethyl-DDC had no effect in this case. Other fungicides were not investigated; the effect of DL-threonine and L-threo-β-phenylserine was not investigated either. However it did appear that DL-alanine in a concentration of 0.10 % had some influence: the severity of disease was reduced to 64 %. It is not impossible that some other amino acids will have a more specific influence than DL-serine which appears to be active in several plant-pathogen combinations. In this connection the investigations of Kuč et al. (1957) on the protective influence of DL-phenylalanine in apples should be remembered.

Experiments with histidine. Histidine occupies a separate place in group A both as concerns structure and activity. Its effect was weaker and much more irregular than that of the amino acids of the serine group. In addition its effect was not influenced by adding fungicides. Because of its fitful results only few experiments were conducted with DL-histidine (table 2). It should be mentioned that D-histidine was active, and L-histidine was not active.

B. Amino acids of the glycine group

In the foregoing it was recorded that the influence of DL-serine or DL-threonine could be increased by combining them with a fungicide. This induced us to reinvestigate the effect of the amino acids which so far had proved to be inactive, in combination with NaDDC in the cucumber seedling test (table 1). Apart from the amino acids already discussed glycine and DL-alanine also exerted a marked influence under such circumstances (fig. 5, table 12).

Glycine and DL-alanine have the formula $COOH.CHNH_2R$, in which R may be substituted by H or CH_3. DL-β-alanine appeared to be phytotoxic. DL-phenylalanine had no effect in contrast to L-threo-β-phenylserine of group A. Nor was an influence found of the amino acids with a longer chain or a ring structure, such as aminobutyric acid, valine, leucine, proline and their isomers. As in the serine-group there was a marked difference in activity between the optical isomers, D-alanine had a far greater effect than L-alanine (table 12). Glycine is optically inactive.

TABLE 12. Effect of glycine and DL-alaninewith and with out NaDDC. Cucumber seedling test.
Effect van glycine en DL-alanine met en zonder NaDDC. Komkommerkiemplantentoets.

	Severity of disease in % *Mate van aantasting in %*	
	Without NaDDC *Zonder* ,,	With NaDDC (100 ppm) *Met* ,, ,,
Control (water)	100	86
Glycine 0.25 %	96	7
DL-alanine 0.25 %	100	21
D-alanine 0.25 %	84	14
L-alanine 0.25 %	93	63

Of the compounds that are active together with glycine and DL-alanine bis-dimethyldithiocarbamylacetic acid and phygon should be mentioned, apart from NaDDC mentioned above. Table 13 illustrates the effect in combination with phygon. Only with 50 ppm there is a pronounced effect.

Intake through the cotyledons gave a result comparable with uptake by the roots, while the effect was also obtained when for instance NaDDC was applied to the cotyledons and the amino acid to the roots.

Related compounds. Apart from the amino acids mentioned above also compounds derived from glycine and alanine were tested, among others some dipeptides. Sarcosine yielded a remarkably good result. Combined with NaDDC

TABLE 13. Effect of combinations of glycine or DL-alanine with phygon. Cucumber seedling test.

Effect van glycine en DL-alanine met phygon. Komkommerkiemplantentoets.

	Severity of disease in % *Mate van aantasting in %*		
	Without phygon *Zonder* ,,	With phygon *Met* ,,	
		20 ppm	50 ppm
Control (water)	100	65	75
DL-alanine 0.25 %	95	95	47
Glycine 0.25 %	100	72	47

50 ppm a 0.10 or 0.05 % solution could reduce the severity of disease to 12 % of the control. In higher concentrations it was phytotoxic. Complete methylation of the amino group reduced the activity; betaine was not active in non-phytotoxic concentrations.

The dipeptides glycylglycine, DL-alanylglycine and glycyl-DL-phenylalanine were also tested, either combined with NaDDC or separately. The first two compounds showed a protective effect but only in combination with NaDDC, the latter being inactive even then, as was also diketopiperazine.

Experiments with other plants. Data on the activity of these amino acids in other plants are still scarce. In broad beans glycine and DL-alanine were inactive, both when applied separately and in combination with NaDDC. This negative result is comparable with that of DL-serine which, when added with NaDDC, did not produce an increased effect either. In tomatoes things were different again, the resistance somewhat increased even when only DL-alanine was applied. It could not yet be established with certainty whether the addition of NaDDC exerted a further influence.

C. Amino acids showing no or a very slight activity

The greater part of the amino acids had no or hardly any effect when they were applied to the roots prior to inoculation. It should be left an open question whether or not they were taken up. As DL-phenylalanine was inactive the results obtained by KUĆ et al. were not confirmed in the plants we tested. A number of amino acids such as DL-α and DL-β-aminobutyric acid, DL-α and DL-β-aminoisobutyric acid, DL-proline, hydroxyproline, DL-norvaline and D-leucine, were harmful to the plants also in low concentrations, to such an extent that the estimation of the disease index became difficult. As a result of the uptake of DL-dioxyphenylalanine the hypocotyls turned black, which also hampered the assessment. Some amino acids such as DL-aspartic acid, and DL-methionine appeared to cause occasionally a decrease in the susceptibility to a slight extent, it is true, but their effect was always far smaller than that of the amino acids of the groups A and B. The experiments were carried out using as much as possible racemates, but in some cases only D- or L-isomers were available. As has been stated above, in the active compounds the D-form is more effective than the L-form, so that negative results obtained with the latter have little value. Threo-β-phenylserine only forms an exeption; the D-form, however, has not been investigated.

The protective effect of some amino acids described above could be explained in the easiest way by assuming that these amino acids are fungitoxic. In the literature some examples are known of growth inhibition of fungi in vitro by amino acids. According to ALTEN & ORTH (1941) the germination of sporangia of *Phytophthora infestans* was inhibited by aspartic and glutamic acids, arginine and some other compounds, but histidine, glycine, hydroxyproline and a few others proved to have a stimulating effect in concentrations of 1 to 0.01 %. ROBBINS & MA (1945) and ROBBINS & MCVEIGH (1946), on the other hand, found, that hydroxyproline inhibited the growth of *Trichophyton mentagrophytes* and some related fungi. However, in general merely the suitability of amino acids as a source of nitrogen or carbon has been investigated. LEONIAN & LILLY (1938) showed that serine could be utilized by a number of fungi; this was confirmed by STEINBERG (1942) and GOTTLIEB (1946). DEDIC-KOCH (1957) established that DL-threonine was a relatively good source of nitrogen for *Cladosporium* n.sp.; histidine could not be utilized, but this held both for D- and L-histidine. NEWTON (1957) found that *Phytophthora parasitica* did not grow in nutrient solutions containing D-alanine, or D-leucine as a source of nitrogen, while L-leucine allowed but little growth. The fungus grew well in solutions containing glycine or L-alanine. However, in these experiments no other source of nitrogen was present so that it cannot be concluded whether an inhibitory action was involved or that the fungus was not able to utilize these compounds.

We investigated the influence of serine and a few other amino acids on the growth of *Cladosporium cucumerinum* and *Aspergillus niger* in the following ways.

Disks of filter paper were soaked in concentrated solutions of amino acids and placed on agar plates seeded with conidia. Use was made of 10, 5, 2.5 and 1 % solutions of DL-serine, DL-threonine, DL-histidine, L-glutamic acid and DL-aspartic acid. In none of the experiments an inhibition zone was observed round the disks. A number of concentric zones appeared, caused by more or less abundant mycelial growth and differences in sporulation. Presumably this phenomenon is due to differences in nitrogen concentration and not to a specific effect of the amino acids, since the same result was obtained when NH_4NO_3 and KNO_3 solutions were used, but not when the disks were immersed in solutions of glucose or sucrose. The germination of the conidia was not inhibited either when the agar was mixed with 0.25, 0.10, or 0.05 % amino acid. The above experiments were only concerned with an inhibiting effect of the amino acids on spore germination. There remained a possibility, however, that mycelial growth would be inhibited in the long run. *Cladosporium cucumerinum* was therefore grown in a nutrient solution containing glucose, sodium citrate and anorganic salts, to which DL-serine had been added in various concentrations from 0 to 1 %. After three weeks the dry weight of the mycelium produced was determined. It showed an increase at higher serine concentrations; but no harmful effect could be established.

In these experiments always DL-serine was used. As in the cucumber test, however, the D-form was active, its influence on mycelial growth was investigated in another experiment. D-serine was added to agar plates, containing glucose, yeast extract and anorganic salts, among which KNO_3. A piece of mycelium of *Cladosporium cucumerinum* was grafted in the centre of each plate. The plates were kept at 23 °C. and the diameter of the colonies was measured daily. D-serine in a concentration of 0.05 % did hardly influence mycelial growth, but the fungus showed little sporulation. Lower concentrations did not seem to have any influence; higher concentrations caused a gradual decrease in growth rate, 0.40 % not allowing any growth at all. *Aspergillus niger* showed a similar sensitivity to D-serine, but the growth of *Fusarium oxysporum* was hardly diminished even by the presence of 0.40 % D-serine. L-serine proved to have no harmful influence. It is remarkable that the toxic effect of D-serine seems to be counteracted by the L-form, DL-serine not being toxic in the concentrations tested.

The influence of DL-serine and glycine in combination with fungicides was also investigated in vitro. Potato-glucose agar mixed with carboxymethyl-DDC was seeded with *Cladosporium* conidia and a filter paper disk, soaked in a 10, 5, 2.5 or 1 % solution of DL-serine or glycine, was placed on the agar. No inhibition of the germination could be observed.

The influence of amino acids and fungicides on the mycelial growth was investigated again by measuring the linear growth on agar plates, containing DL-serine or glycine and NaDDC, TMTM or TMTD in various concentrations from 0.1–10.0 ppm. The growth inhibition caused by the fungicides did not appear to be influenced by the presence of these amino acids. The influence of D-serine was not investigated separately.

DISCUSSION

In the foregoing we have demonstrated that the susceptibility of cucumber plants to *Cladosporium cucumerinum* decreased when prior to or shortly after inoculation certain amino acids were applied to the plants. The active amino acids could be grouped into two categories, viz. those which were effective by themselves, and whose activity could be increased by the application of a fungicide at the same time, in particular a thio- or dithiocarbamate; and those which had an influence only in combination with such a fungicide. These results could be confirmed in some other plant-pathogen combinations.

The expressions "reduction of the severity of disease", "decreased susceptibility", "increase in resistance" etc. have been used alternatively. These were intended only to describe the phenomena observed. At the moment it is difficult to give an explanation of the results obtained. We must therefore restrict ourselves to a discussion of the various possiblities.

It should be mentioned first that the serine content of cucumber seedlings, which had stood in a solution of DL-serine, appeared to increase greatly. The same held for DL-threonine and glycine; it has not been investigated for other amino acids. It may therefore be assumed that at any rate the amino acids mentioned are taken up as such.

As far as the activity of the amino acids of group A is concerned, there are the following possibilities. 1. The amino acids affect the metabolism of the plant in some way resulting in an increase in the resistance without there being a direct toxic effect on the fungus. 2. The amino acids exert a direct toxic influence on the parasite in the plant. 3. The amino acids are either themselves converted into a fungitoxic compound or they influence the metabolism of the plant in such a way that a fungitoxic compound is produced.

Too little is known about the causes of resistance and susceptibility to comment much on the first statement. In connection with the importance of serine as a methyl group donor we used a number of compounds in the cucumber seedling test, of which is known that they can act in a similar way. However, xanthine, adenine, guanine nor uracil appeared to have an influence on the resistance. It stands to reason that this should not lead to the conclusion that the effect of serine is due to some other property. In the literature it is stated that the growth of seedlings is inhibited by various amino acids (AUDUS & QUASTEL, 1947; STEINBERG, 1949; FRIES, 1951; NEWTON, 1957). According to some authors (KEYWORTH & DIMOND, 1952; DAVIS & DIMOND, 1952) the resistance of tomatoes to *Fusarium oxysporum* increased as a result of mechanical or chemical injury of the roots. From table 1 it is apparent, that in various cases a phytotoxic effect was established, although at first sight there seemed to be little correlation between the toxic effect of the amino acids and their chemotherapeutic activity. In these experiments, however, we had no accurate way of measuring the harmful effect, so we conducted some preliminary experiments on the influence of amino acids on the root growth of cucumber seedlings. For this purpose, three-day old seedlings were used. By that time the cotyledons had not yet been developed. The plantlets were placed on filter paper which had been moistened with a certain volume of solutions of different amino acids. DL-serine, DL-threonine and DL-histidine indeed caused a strong inhibition of the growth of the main root. The D-isomer appeared to be the most active in this case, 0.01 M D-serine for instance gave an increase in length of 11 % of that of the control; L-serine (also 0.01 M) of 88 %. The effect of the D-form was counteracted slightly by the L-isomer, the DL-acids still showing a marked inhibitory action. These results are in contrast to those obtained with fungi. NEWTON (1947) obtained the same results in his investigation on the influence of alanine on the growth of wheat seedlings. However, we did not find a correlation between this growth inhibition and the resistance increasing effect of the amino acids. For instance, L-threo-β-phenylserine appeared to inhibit the root growth slightly, while DL-glutamic acid and D-alanine had this effect to a high degree. Addition of NaDDC did not increase the effect exerted by glycine or DL-serine. These results are not in agreement with the observations on the phytotoxic activity of the compounds as mentioned in table 1 and those described in the third section. This must be either a result of the different conditions or an entirely different effect may be involved here. At any rate it appears that this inhibition of root growth cannot be taken as the only criterium of the phytotoxic effect.

The second point was mentioned already in the previous section. In vitro a toxic effect could be demonstrated only when using D-serine in fairly high concentrations (0.005 M or more) without L-serine being present. However, as the conditions within the plant are quite different we cannot exclude this possibility.

In order to investigate the third possibility more closely a number of experiments was carried out, whereby plants were treated with amino acids in the usual way, thereupon they were rinsed, frozen and squeezed. Disks of filter paper were moistened with the sap thus obtained, dried at ± 30 °C., moistened again etc. until 0.5 ml sap had been added. This sap contained both vacuolar sap and protoplasm. The disks were then placed on agar plates seeded with conidia of *Cladosporium cucumerinum*, *Aspergillus niger* or *Glomerella cingulata*. In several experiments (but not in all of them) in which sap of roots or hypocotyls of plants, treated with DL-serine had been used an inhibition zone could be observed. Sap of control plants never gave any inhibition. So it seems that under the influence of the treatment a fungitoxic substance can be produced in the plant. As this could not be shown in all the cases and since the inhibition was fairly small, it cannot be concluded that the decreased susceptibility of the plants is due to this or whether a secondary effect is involved. Similar experiments were also conducted with plants that had been placed in a solution of amino acids and fungicides (mainly NaDDC and TMTD). In this case a fungitoxic substance was always to be found in sap obtained from the roots; in sap derived from the hypocotyls it was found occasionally. However, it was conspicious that the inhibition, and apparently the concentration of the fungitoxic compound in the sap, was greater when the fungicide had been applied to the plants in combination with an amino acid (table 14). We have never found a fungitoxic effect in sap derived from cotyledons.

TABLE 14. Fungicidal action of expressed sap from plants, treated in solutions of glycine and glycine and NaDDC. Test fungus *Glomerella cingulata*.
Fungicide werking van perssap van planten, behandeld met glycine of glycine met NaDDC. Testschimmel Glomerella cingulata.

	Diameter inhibitionzone in mm *Diameter remmingszone in mm*			
	Plants treated with *Planten behandeld met*			
	Water	NaDDC (100 ppm)	Glycine (0.25 %)	NaDDC + Glycine (100 ppm) (0.25 %)
Cucumberseedlings (hypocotyls) .	–	–	–	18
Lupins (stems)	–	14	–	21

A few experiments were carried out using other plants. Sap of the stems of lupins, which had stood in a solution of glycine and NaDDC caused a larger inhibition zone than that of plants treated with NaDDC only (table 14). The same held true for the stems of French beans. Sap of the leaves of lupin, French beans, wheat and barley, stems and leaves of peas and broad beans gave negative results, after treatment of the plants via the roots.

When table 14 is considered the question arises whether the uptake and/or the transport of the fungicide might increase under the influence of glycine. VAN RAALTE (1952) demonstrated that NaDDC and TMTD, in contrast to TMTM, were not transported through a section of a potato petiole. When in the test used by VAN RAALTE a mixture of NaDDC and one of the active amino acids was used, no transport occurred either. Using sections of hypocotyls of cucumber seedlings, previously treated with DL-serine, we did not obtain a positive result either. It is likely, however, that this is a question of diffusion, so that this case is not directly comparable with transport through an intact plant.

In cucumber plants NaDDC seems to be taken up and transported upwards to some extent, even if its protective effect is but slight. It might be that this process was furthered by amino acids. The fact that in particular fungicides produced an effect in combination with serine etc. while related, but nonfungitoxic compounds were inactive, might point in this direction. Carboxymethyl-DDC is an exception; as already stated, however, it is possible that here, too, a fungitoxic ion is produced. Reversely, fungicides of a different structure produced a similar effect. In addition the phytotoxicity of the fungicides increased in the presence of amino acids, which may be the result of an increased uptake. It should be mentioned that carbohydrates, such as glucose and sucrose, which might influence the uptake through the roots, had no effect. It is remarkable, however, that a good effect was obtained, when the fungicide was applied to the cotyledons and the amino acids to the roots. On the other hand, seedlings whose roots had been cut off, were also protected by DL-serine and NaDDC to a higher extent than when only DL-serine was applied. The latter seems an indication, that it must be a matter of an effect on the transport rather than on the actual uptake process.

According to this view the activity of the amino acids of group A is twofold: they have an increasing effect on the resistance and also influence the transport of various fungicides. The latter property is shared by the amino acids of group B.

On the other hand it is also imaginable that not the amino acids influence the effect of the fungicides, but that the latter influence the effect of the amino acids. The difference between the compounds of group A and B would be only a relative one in this case: that is, the effect of glycine and DL-alanine is too slight to be perceptible and it only becomes observable when it is being corroborated by the presence of, for instance, NaDDC. However, it is difficult to imagine how this corroboration may be brought about. It has been shown that zinc dimethyldithiocarbamate caused a change in the amino acid composition of leaves and roots of sugar beets (FULTS et al. 1951, BLOUCH et al. 1952). It is therefore possible that some conversions of serine etc. are being furthered or inhibited under the influence of NaDDC. It is less understandable, however, that the various fungicides have a similar effect. The process involved must have little specificity. In the second place it is possible, that the fungicides influence the uptake of the amino acids in some way. In this connection the work of BANDI (1957) should be mentioned. He found that the roots of peas took up more sucrose under the influence of streptomycine. Streptomycine appeared to cause an increase in the permeability of the tissue. According to REINHOLD & POWELL (1956) the uptake of glycine and glutamic acid in hypocotyls of sunflower seedlings was increased under the influence of indole-3-acetic acid. Carboxymethyl-DDC, as has been shown by VAN DER KERK et al. (1955) acts as a growth regulator. However, we obtained no results after adding indole-3-acetic acid to the medium, neither in case of applying it separately or in combination with amino acids or a fungicide.

This result is also important in connection with the investigations of DAVIS & DIMOND (1953), who found that tomatoes, treated with plant growth regulators showed an increased resistance to *Fusarium oxysporum*, presumably as a result from changes in the metabolism of the plants.

A question which should be discussed in more detail is, that the D-isomers appeared to be more active than the L-forms. Various investigations have shown that optical isomers of amino acids have a different effect on plants. It has been mentioned already that NEWTON found a difference in phytotoxicity to wheat seedlings, D-alanine being toxic, while L-alanine proved to be a suitable source of nitrogen. This was confirmed in our own experiments. BIRT & HIRD (1956) established that carrot disks took up L-histidine more rapidly than D-histidine.

EL-SHISHINY & NOSSEIR (1957) obtained similar results with glutamic and aspartic acids. The relatively small uptake of the D-isomer could be one of the reasons, why comparatively high concentrations of the amino acids had to be applied. In the experiments of EL-SHISHINY & NOSSEIR it appeared also that D-glutamic acid was accumulated, while L-glutamic acid seemed to be assimilated rapidly. The ineffectiveness of L-amino acids in our experiments is possibly a result of their rapid conversion. L-threo-β-phenylserine was an exception: however, it is imagineable, that in this compound also the L-isomer is being assimilated less rapidly than in the case of serine and threonine, which play an active role in the metabolism. If this argument should be correct, it follows that serine etc. themselves are active and not a derivative.

It will be necessary to collect more data on the uptake of amino acids and their influence on the metabolism of plants before we will be able to formulate a hypothesis on their role in the increase in the resistance described.

SUMMARY

The influence of amino acids on the resistance of cucumbers to *Cladosporium cucumerinum* was investigated in seedlings and older plants. The majority of the amino acids tested appeared to have no effect; but treatment of the plants with DL-serine, DL-threonine, L-threo-β-phenylserine and DL-histidine before inoculation produced a marked decrease of the susceptibility of the plants. A further reduction could be obtained when these amino acids were applied in combination with a non- or somewhat systemic fungicide, in particular a thio- or dithiocarbamate.

Besides the four amino acids already mentioned also glycine and DL-α-alanine and some related compounds appeared to have a favourable effect when applied in combination with a fungicide. The D-isomers proved to be active, the L-isomers had little effect. In seedlings the protective effect could be maintained for about a week. It did not matter whether the compounds were applied to the roots or cotyledons, respectively leaves. Also when the treatment took place 24 hours after inoculation a considerable effect was demonstrated.

In some other plants, such as broad beans and tomato plants, various amino acids, in particular DL-serine, had a favourable effect against *Botrytis fabae* and *Phytophthora infestans* respectively. In the case of broad beans no marked influence was found by the application of a fungicide and amino acid at the same time; in tomatoes a favourable effect was obtained.

The influence of some amino acids on the growth of fungi in vitro was studied. In addition some experiments were conducted on the occurrence of fungitoxic compounds in sap of treated plants, and on the influence of several amino acids on the growth of cucumber seedlings.

SAMENVATTING

Naar aanleiding van een onderzoek van VAN RAALTE, KAARS SIJPESTEIJN, VAN DER KERK, OORT en PLUYGERS, waarbij gebleken was, dat het in vitro weinig fungicide S-carboxymethyldimethyldithiocarbamaat (carboxymethyl-DDC) een systemische werking had tegen *Cladosporium cucumerinum* bij komkommers, werd nagegaan, of de werking van deze verbinding beïnvloed werd door amino-

zuren. De meeste proeven werden uitgevoerd met de komkommerkiemplantentoets, waarbij kiemplantjes gedurende twee dagen in een oplossing van de te onderzoeken verbinding werden geplaatst, vervolgens overgebracht in water en geïnoculeerd. Daarnaast werden enkele proeven gedaan op komkommerplanten, waarbij zich enkele bladeren hadden ontwikkeld en die eveneens met *Cladosporium cucumerinum* werden geïnoculeerd. Als objecten werden verder gebruikt tuinbonen, geïnoculeerd met *Botrytis fabae*, en tomaten, geïnoculeerd met *Phytophthora infestans*. In deze gevallen werden de verbindingen als oplossingen of aan de wortels of door bespuiting aan de bladeren toegediend. Inoculatie had gewoonlijk na de behandeling plaats door bespuiting met een suspensie van conidiën respectievelijk zoösporen. De planten werden vervolgens in een vochtige ruimte gehouden tot bij de controle een duidelijke aantasting was opgetreden. De mate van aantasting van de behandelde projecten is aangegeven als percentage van de aantasting van de controle.

In de komkommertoets werd een groot aantal aminozuren onderzocht. Hierbij bleek, dat ze naar hun werking in drie groepen waren in te delen (tabel 1):

A. Aminozuren, die op zichzelf de vatbaarheid verminderden en waarvan de werking versterkt kon worden door toevoeging van carboxymethyl-DDC of een niet of weinig systemisch werkend fungicide als natriumdimethyldithiocarbamaat (NaDDC). B. Aminozuren, die op zich zelf geen invloed hadden op de vatbaarheid, maar wel in combinatie met een fungicide als NaDDC. C. Aminozuren, die op zichzelf toegepast of in combinatie met een dergelijk fungicide geen of slechts zeer geringe werking hadden.

Tot de eerste groep behoorden DL-serine, DL-threonine, L-threo-β-phenylserine en DL-histidine (tabel 2). Van de stereoisomeren was de D-vorm veel werkzamer dan de L-vorm (tabel 3). De werkzaamheid van deze verbindingen bleek niet terug te brengen op een functie als stikstofbron. Eenzelfde gunstig effect werd verkregen als ze aan de wortels of aan de cotylen werden toegediend (tabel 4), terwijl de beschermende werking een week na de behandeling nog niet bleek te zijn afgenomen (tabel 8). Over het algemeen werd een belangrijke reductie van de aantasting pas vastgesteld, als oplossingen van 0,5 tot 1 % werden gebruikt; werd daaraan echter carboxymethyl-DDC of NaDDC toegevoegd, dan waren concentraties van 0,25 tot 0,10 % voldoende (tabel 5 en 6; fig. 1 en 2). Andere fungiciden als captan en phygon bleken een dergelijk, zij het wat zwakker, effect te geven (tabel 7).

Bij oudere planten werd eveneens een gunstig effect verkregen door de wortels in oplossingen van DL-serine of DL-serine met NaDDC te plaatsen of de bladeren met deze oplossingen te bespuiten (tabel 9). Ook wanneer de planten 24 uur na de inoculatie hiermee werden bespoten kon de aantasting aanzienlijk verminderd worden (fig. 3 en 4). Bespuiting 48 uur na inoculatie had geen effect.

Bij tuinbonen, gedurende twee dagen in oplossingen van DL-serine of DL-threonine geplaatst en vervolgens geïnoculeerd, trad een vermindering van het aantal door *Botrytis fabae* veroorzaakte vlekken op (tabel 10). Toevoeging van NaDDC of een ander fungicide had hierop geen effect.Bij tomaten werd door bespuiting van de bladeren met oplossingen van DL-serine en DL-serine met NaDDC de aantasting gereduceerd tot respectievelijk 70 % en 46 % van de controle (tabel 11).

Tot groep B behoren glycine en DL-alanine. Met NaDDC of phygon gecombineerd gaven deze gunstige resultaten in de komkommertoets (tabel 12 en 13;

fig. 5); alleen of met carboxymethyl-DDC bleken ze echter vrijwel onwerkzaam. Hetzelfde geldt ook voor sarcosine en enkele dipeptiden als glycylglycine en DL-alanylglycine (tabel 1). Met aminozuren van deze groep werden slechts enkele proeven gedaan bij andere objecten. Bij tuinbonen werden geen positieve resultaten bereikt. Bij tomaten bleek DL-alanine alleen reeds een afname van de aantasting te veroorzaken.

De invloed van verschillende aminozuren op de sporenkieming, myceliumgroei en drogestof productie in vitro werd nagegaan bij *Cladosporium cucumerinum* en *Aspergillus niger*. Een concentratie van 1 % DL-serine bleek nog geen ongunstig effect te hebben. Bij aanwezigheid van alleen D-serine in het medium bleek de myceliumgroei wel te worden geremd, doch pas bij een tamelijk hoge concentratie.

In sommige gevallen kon in perssap van wortels en hypocotylen of stengels van behandelde planten een fungitoxische stof worden aangetoond (tabel 14). Of deze een rol speelt bij het beschreven effect op de aantasting der planten is nog niet te zeggen. Een verklaring van deze vermindering van de aantasting of verhoging van de resistentie kan nog niet gegeven worden, maar enkele mogelijkheden worden besproken.

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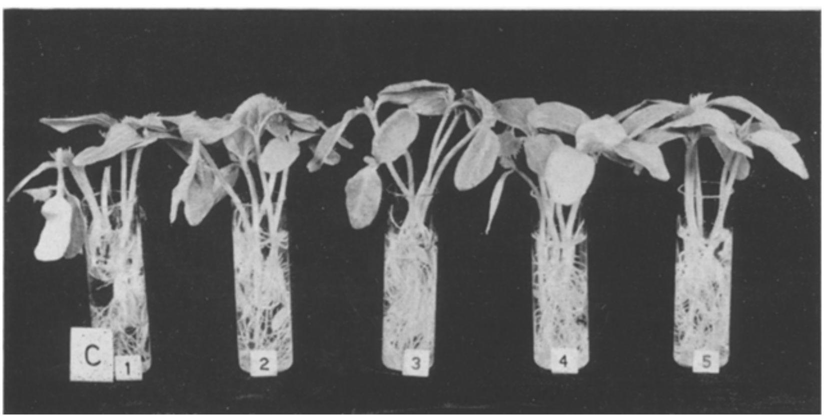
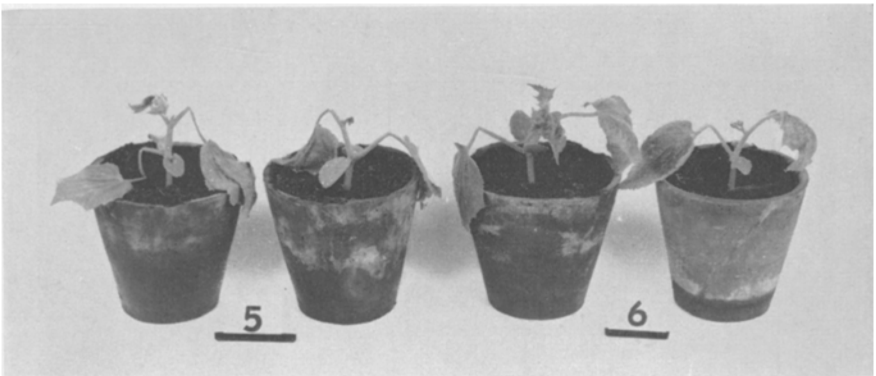


FIG. 1 and 2. Influence of DL-serine on the resistance of cucumber seedlings to *Cladosporium cucumerinum*. Concentrations of DL-serine used: from left to right 0, 0.10, 0.25, 0.50 and 1.00%. To all solutions used in fig. 2 100 ppm carboxymethyl-DDC had been added.

Invloed van DL-*serine op de resistentie van komkommerkiemplanten tegen* Cladosporium cucumerinum. *Concentratie van* DL-*serine*: *v.l.n.r. 0, 0,10, 0,25, 0,50 en 1,00%. Aan alle oplossingen van fig. 2 was 100 dpm carboxymethyl-DDC toegevoegd.*



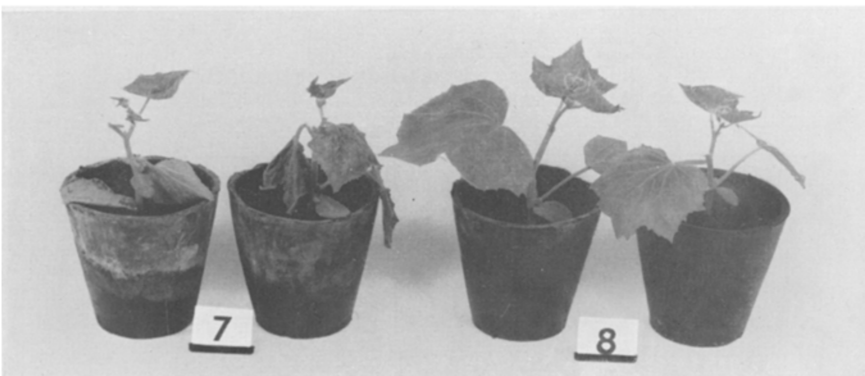


FIG. 3 and 4. Cucumber plants sprayed with various solutions 24 hours after inoculation with *Cladosporium cucumerinum:* 5, water; 6, DL-serine 0.25 %; 7, NaDDC 100 ppm; 8, DL-serine, 0.25 % and NaDDC, 100 ppm.

Komkommerplanten 24 uur na inoculatie met Cladosporium cucumerinum *bespoten met verschillende oplossingen, n.l.: 5, water; 6, DL-serine, 0,25 %; 7, NaDDC 100 dpm; 8, DL-serine 0,25 % en NaDDC, 100 dpm.*

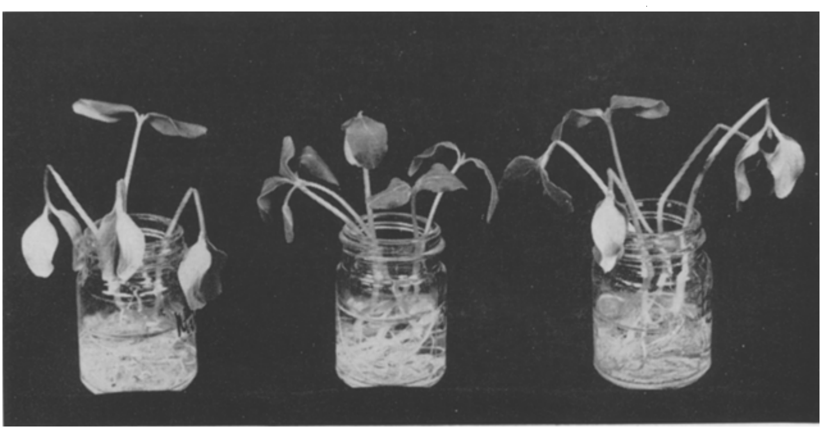


FIG. 5. Effect of glycine and NaDDC in the cucumber seedling test. Plants treated with: 100 ppm NaDDC (left), 100 ppm NaDDC and 0.25 % glycine (middle), 0.25 % glycine (right).

Effect van NaDDC en glycine in de komkommerkiemplantentoets. Planten behandeld met: 100 dpm NaDDC (links), 100 dpm NaDDC en 0,25 % glycine (midden) en 0,25 % glycine (rechts).

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