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THE IMPORTANCE OF AMINO ACIDS FOR THE DEVELOPMENT OF FUSARIUM OXYSPORUM F. LUPINI SN. ET H. IN THE XYLEM OF LUPINS

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INTRODUCTION

Fusarium oxysporum f. *lupini* is the cause of the wilting disease of lupins. The fungus develops primarily in the xylem vessels of roots and stem. Since the xylem sap of herbaceous plants usually does not contain sugars or other organic substances (KRAMER, 1949), the question arises from where the fungus derives its organic substrates. This problem may be of some importance for the study of the causes of the resistance which certain varieties of lupins have developed against this disease. It has been shown that development of the fungus is inhibited in resistant plants, although some growth is possible.

ZEEVAART (1955) found aspartic acid in the exudation sap of *Lupinus luteus* L., and suggested that this could be used as a source of carbon; SAALTINK (unpublished results) showed later that glutamic acid, asparagine, alanine and probably leucine were also present, aspartic acid being quantitatively the most important. POTAPOV and CSEH (1955) demonstrated the presence of several amino acids in the sap of maize and pumpkin.

It is known that *Fusarium oxysporum* f. *lycopersici* is able to grow in a medium containing no other organic substances than a single amino acid. (GOTTLIEB, 1946). However, the amounts Zeevaart found in exudation sap were considerably less than those used in Gottlieb's experiments.

In the experiments described below the growth of *Fusarium oxysporum* f. *lupini* was determined in solutions of different amino acids in varying concentrations. Special attention has been given to the effect of those amino acids that have been found in the exudation sap of lupins, and to the influence of the concentration of the amino acids.

MATERIAL AND METHOD

Richards' solution containing 10 gm KNO_3, 5 gm KH_2PO_4, 2.5 gm $MgSO_4$. 7 aq, a trace $FeCl_3$ in a litre distilled water was used as a basic solution to which organic substances were added in different concentrations.

The pH of the solution was 4.1. When amino acids had been added the pH was adjusted by means of potassiumhydroxyde.

The fungus was grown in 100 ml erlemeyers containing 25 ml of the solution. Larger quantities of liquid were put in proportionally larger erlemeyers in order to maintain a more or less constant proportion between volume and surface. The solutions were inoculated either by means of small disks which had been punched into an agarplate which was homogeneously covered by the fungus, or by adding 0.5 ml of a spore suspension obtained from a "shaking" culture. The agar disks themselves allowed some growth, so that the controls (no organic substance present) yielded dry weights of 10–30 mg. The erlemeyers were incubated at 25° C. for 7–10 days, in undisturbed cultures, but the flasks were shaken once or twice a day. At the end of the incubation period the mycelia were filtered and washed with dilute nitric acid. They were then washed several times with distilled water, dried and weighed. The nitric acid was used to remove a precipitate that was formed in the media when the pH rose above 7. The pH of the filtrate was determined. The experiments were carried out in duplicate or triplicate. The tables and figures give the average values.

The fungi that were used for the experiments comprised several strains of *Fusarium oxysporum* f. *lupini* isolated by G. J. SAALTINK, Laboratory of Phytopathology, Wageningen.

Several experiments were repeated with a strain of *Fusarium oxysporum* f. *callistephi*. The results were the same.

RESULTS AND DISCUSSION

F. oxysporum f. *lupini* proved to be able to grow in a medium containing 0.5 % aspartic acid, glutamic acid, ornithine, arginine, glycine or alanine. The yield of dry weight decreased from aspartic acid to alanine in the order mentioned above. The former two amino acids allowed an even better growth than 0.5 % glucose controls. In solutions of leucine, valine and phenylalanine, however, dry weights were found which surpassed those of the controls (i.e. solutions lacking organic substances) only by a few milligrams.

An identical series of experiments was carried out with 1 % glucose added to the solutions. None of the added amino acids had a toxic effect; all of them, even leucine and valine promoted growth as compared with the control solutions containing glucose only (both 1 and 1.5 %).

It is of little use to compare the nutritional value of the amino acids and of glucose or sucrose, because the added amounts are difficult to compare. In these experiments we added 0.5 % organic substances as did GOTTLIEB (1946) in his investigations. This means, however, that there are great differences in concentration. As will be shown later, the concentration of amino acid is very important for its utilization. In another experiment 0.035 M of every amino acid was added. In this case arginine proved to be a better substrate than

aspartic acid, but growth on glucose and sucrose alone was about twice as great as on the arginine.

The importance for growth of aspartic acid, glutamic acid, asparagine, alanine and leucine was examined in greater detail.

Fig. 1 shows the relation between growth of the fungus and the concentration of aspartic acid in the medium. 0.075 M. aspartic acid appeared to yield maximal growth; a further increase in the concentration of aspartic acid resulted in a reduction of the dry weight; in some cases it was reduced almost to zero. Growth did not start at concentrations below 0.0125 M.

When the medium contained both glucose and aspartic acid the relation between growth and amino acid concentration was more or less linear. The same results were obtained when nitrate was omitted

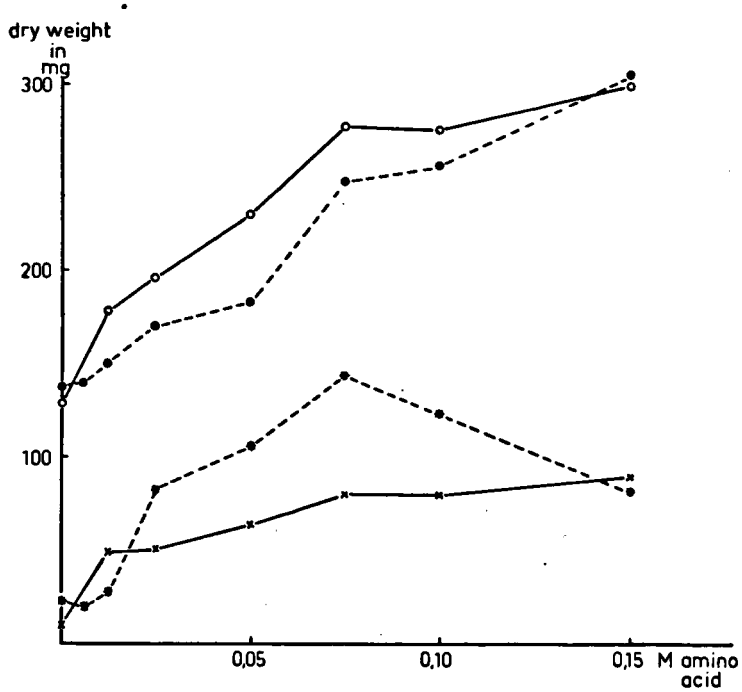

Fig. 1. Relation between growth and amino acid concentration of the medium. *------* aspartic acid; ●------● aspartic acid and 0.05 M glucose; ×———× glutamic acid; ○———○ glutamic acid and 0.05 M glucose.

from the solution. It is probable therefore that aspartic acid is being used as a source of both carbon and nitrogen.

The pH of the medium increased, especially when no glucose was present.

Glutamic acid gave somewhat different results (Fig. 1). Growth began to increase with increasing concentration. Increasing the concentration above 0.075 M, however, did not result in any further

increase in dry weight production. This held true both for solutions with and without glucose. Addition of more glucose did, however, increase the growth. Apparently some factor is lacking which is specifically necessary for the utilization of glutamic acid. We did not succeed in removing this restriction by adding trace elements, especially zinc and iron, which appeared to be very important when glucose was used as source of carbon. A solution of vitamins containing riboflavin, nicotinic acid amide, p-aminobenzoic acid, pyridoxine, aneurine and biotin or diluted expressed sap of lupins did not have any effect either.

Asparagine proved to be a rather unsuitable substrate (Table 1). Larger amounts of asparagine promoted growth, but only if at least

TABLE 1

The effect of asparagine on the dry weight production in mg.

Asparagine \ Glucose	0.00 M	0.10 M	0.25 M
0.150 M	31.7	93.7	273.6
0.100 „	31.2	128.1	257.4
0.075 „	23.5	119.7	169.0
0.050 „	22.3	127.1	128.7
0.025 „	36.1	120.6	135.0
0.013 „	25.1	106.3	126.1
0.000 „	18.2	98.1	101.9

0.25 M glucose was present in the medium. It is to be questioned whether this increase in growth can be ascribed to the effect of the asparagine itself. It might be possible that the asparagine is acting as a source of nitrogen, although on account of former experiments the amount of potassium nitrate present is supposed to be sufficient. The presence of trace elements in the asparagine is another possible explanation. The growth in a glucose medium is very sensitive to the addition of trace elements; this has been mentioned before.

In Table 2 it is shown that alanine and leucine were not very good sources of carbon either.

TABLE 2

Leucine and alanine as a source of carbon for *Fusarium oxysporum* f. *lupini*. Dry weights in mg.

Leucine \ Glucose	0.00 M	0.20 M	Alanine \ Glucose	0.00 M	0.20 M
0.075 M	52.7	135.3	0.075 M	14.4	203.0
0.050 „	18.7	138.4	0.050 „	4.5	197.3
0.025 „	11.6	72.3	0.025 „	0.0	169.3
0.013 „	2.5	93.6	0.013 „	0.0	157.2
0.006 „	0.0	69.5	0.006 „	0.0	174.0
0.000 „	0.0	98.6	0.000 „	0.0	155.7

It is known that mixtures of amino acids often constitute a better substrate than solutions containing a single amino acid. The fungus grew very well in a medium containing Difco Bacto casaminoacids as is demonstrated in Table 3.

There was never any inhibition of growth, but an increase in concentration above 4.3 % increased the dry weight only when the medium contained more than 0.05 M glucose. This is the same phenomenon we were confronted with in the experiment described in Table 1.

TABLE 3

Effect of casaminoacids on growth in the presence and absence of glucose. Dry weights in mg.

Casaminoacids \ Glycose	0.00 M	0.05 M	0.20 M
6.30 %	172.7	223.8	429.5
4.73 „	186.6	237.1	319.8
3.15 „	151.4	136.8	200.6
1.58 „	110.5	76.0	161.3
0.79 „	64.3	100.2	191.1
0.39 „	35.3	93.8	181.2
0.00 „	26.3	54.8	142.1

These solutions contain 0.10; 0.075 etc. M. glutamic acid.

Mixtures of two or more amino acids in varying proportions (aspartic acid/glutamic acid; asparagine/glutamic acid; aspartic acid/glutamic acid; asparagine/alanine/leucine; etc.) yielded higher dry weights than any one of the components alone. A solution containing 0.050 M glutamic acid 0.0015 M aspartic acid, 0.0024 M leucine and 0.0020 M alanine produced 89.1 mg dry matter. However a solution of 0.05 M glutamic acid alone produced only 31.8 mg., and a 0.10 M solution of glutamic acid 59.7 mg. The same solutions diluted four times (0.0125 M. glutamic acid, etc.) did not allow any growth at all.

Thus we have found, in agreement with the results of ANDERSON and EMMART (1934) and GOTTLIEB (1946) with *Fusarium oxysporum* f. *lycopersici*, that *Fusarium oxysporum* f. *lupini* is capable of using various amino acids as its only source of carbon. The most favourable concentrations were found between 0.050 and 0.075 M. There was relatively little increase in growth at higher concentrations. We must conclude that under our experimental conditions some factor essential for the utilization of some amino acids seems to be lacking.

From the figures and tables presented, it can be seen that perceptible amounts of mycelium are usually found at concentrations of 0.0125 M. and higher. ZEEVAART (1949) estimated the aspartic acid concentration of exudation sap of non-inoculated lupins at about 0.02 % or 0.0015 M. In the sap of inoculated plants the concentration was still less. It is probable that the liquid in the xylem vessels of intact transpiring plants is even more dilute. On the other hand amino

acids may continuously be given off to the vessels so that the concentration of the sap is being kept more or less constant, while in our experiments the concentration is gradually decreasing.

Table 4 shows the results of an experiment in which 100, 50, 25 and 12.5 ml of different solutions of aspartic acid were inoculated with the same amount of fungus. The lowest concentration — 0.0125 M. — did not allow any growth in the flasks containing 12.5 ml., but 50 ml yielded some mycelium, and 100 ml. allowed good growth.

TABLE 4

Effect of quantity and concentration on the utilization of aspartic acid

	100.0 ml	50.0 ml	25.0 ml	12.5 ml
0.050 M	448.3	216.7	170.2	72.1
0.025 „	319.8	193.3	131.9	64.8
0.013 „	112.9	76.2	51.0	42.2
0.000 „			40.5	

However, doubling the concentration has a much greater effect than doubling the quantity when low concentrations are used.

In another experiment 50 mg of aspartic acid was added to erlemeyers respectively containing 100, 50, 25 and 12,5 ml of Richards' solution. The amino acid concentration ranged from 0.05 to 0.4 %. The first and second solution yielded dry weights of 33.2 and 32 mg, the third 44.2 mg and the fourth — the highest concentration — 84 mg. This shows very clearly that very low concentrations cannot be compensated for by greater quantities of the solution. A solution containing less than 0.1 % aspartic acid is apparently too greatly diluted for the fungus to take up enough of the substance.

It therefore seems unlikely that the amino acids in the xylem sap of lupins are very important as a source of carbon for *Fusarium*. It is more probable that the fungus draws the necessary organic substances from the adjacent living cells. This conclusion is supported by the following experiment. Lupins were decapitated, and the exudation sap was collected during twenty four hours. When 25 ml of this sap was inoculated, and incubated in the usual way the yield was 3.5 mg. Dry weights of 200–300 mg were found when glucose had been added to the sap. There was no indication of the presence of an inhibiting factor, even in the sap of resistant plants, but it was merely the lack of organic substrates which seemed to prevent growth.

We may add here that SANWAL (1956) found that *F. oxysporum* f. *lycopersici* developed mycelium in a medium of aspartic or glumatic acid, although the organism was unable to produce toxins under these conditions.

SUMMARY

Fusarium oxysporum f. *lupini* has been shown to grow in media with one or more amino acids as the only source of carbon. Growth, however, was usually less than in equimolar solutions of sugar. Particular attention was given to the importance of aspartic acid, glutamic acid, asparagine, alanine and leucine. The three latter

substances proved to be rather unsuitable substrates for this fungus, although it is possible that some factor essential for their utilization is lacking in these experiments, as must be the case for glutamic acid. Aspartic acid became toxic in concentrations of about 0.1 M.

The minimal concentration allowing growth, was shown to be about 0.0125 M. The disadvantages of a low concentration could not be compensated for by greater quantities of the diluted solution.

Comparing the results with the data known about the presence and concentration of amino acids in the xylem vessels of lupins we must conclude that these substances cannot be very important for the development of *Fusarium* in the xylem vessels.

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