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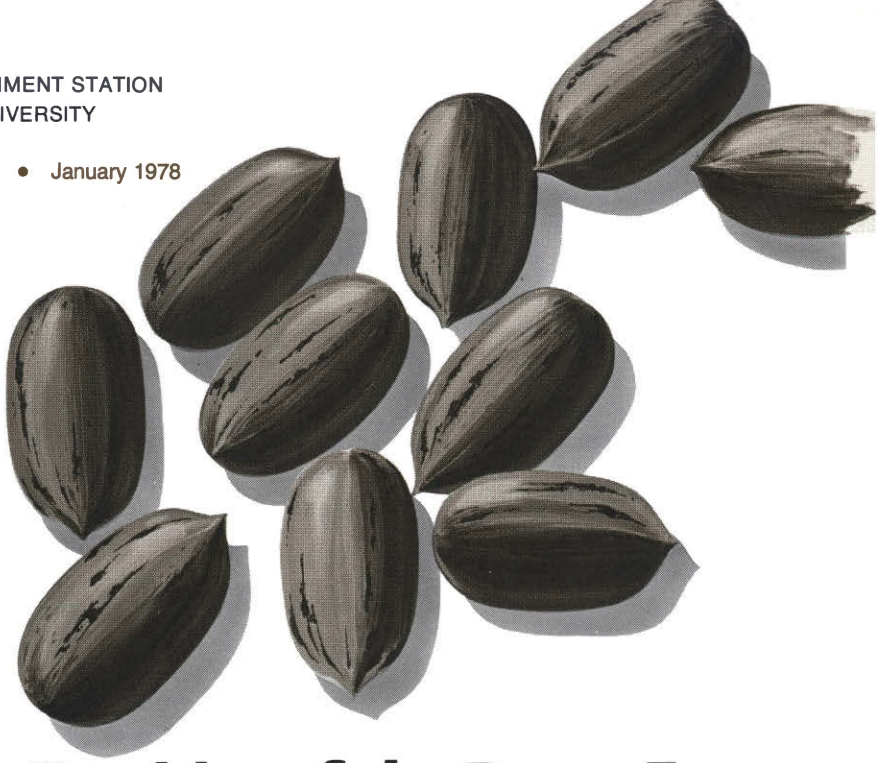

Nitrogen Nutrition of the Pecan Fungus, *Fusicladium effusum* WINT.

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NITROGEN NUTRITION OF THE PECAN SCAB FUNGUS, *FUSICLADIUM EFFUSUM* WINT.[1]

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Pecan scab, incited by the fungus *Fusicladium effusum* Wint., is a major limiting factor in the commercial production of nuts from susceptible cultivars, seedlings and native trees of the pecan (*Carya illinoensis* [Wang.] Koch) when rains and extended dew periods are frequent. Primary lesions develop in the spring and early summer on twigs, nuts and leaflets from infections by conidia released from overwintering stromata on twigs and nut shucks. Secondary infections are caused by conidia produced on primary lesions. If scab is not controlled, entire crops may be lost or greatly reduced in quality.

Through continued field testing, fungicide spray programs are being developed and improved. However, very little research has been conducted on the physiological interrelationships between the scab fungus and its host, the pecan. A body of information needs to be developed so as to gain some insight into such relationships so that the disease will be better understood and better control measures developed.

The author has performed research on the carbohydrate and nitrogen nutrition of the fungus. The work on carbohydrate nutrition has been reported by Barnes and Adams (3) and Hopp and Barnes (10). This bulletin reports results of research performed on nitrogen nutrition of the fungus.

Part I
Inorganic Nitrogen Sources

Nitrogen is an essential element that is used by fungi for functional as well as structural purposes. "Not all nitrogen sources are equally suitable for all fungi. Fungi may be specific in the nitrogen sources they utilize" (12). The choice of an inorganic nitrogen source for fungus nutrition studies with a

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chemically-defined (synthetic) medium is sometimes a difficult one. Cochrane (7) and Lilly and Barnett (12) have stated that many fungi preferentially utilize nitrate compounds over ammonium compounds. Nitrite and cyanide compounds are very toxic to almost all fungi, particularly in acid media, because of the formation, respectively, of HNO_2, very destructive to proteins and amino acids, and HCN, an enzyme poison (7).

The inorganic nitrogen requirements of the pecan scab fungus, *Fusicladium effusum* Wint., have not been extensively investigated. Davis (8) used NH_4NO_3, $(NH_4)_2SO_4$, $Ca(NO_3)_2$, $NaNO_3$ and KNO_3 in a limited study with an agar-solidified synthetic medium at a near neutral pH. Of these compounds, $NaNO_3$ and KNO_3 supported the most growth. NH_4NO_3 followed closely. The relative utilization of many nitrate, ammonium, and nitrite compounds by *F. effusum* at acid and near-neutral pH levels is reported here. A preliminary report has been published (2).

Materials and Methods

Agar Plate Tests

Twenty-two chemically pure or reagent grade inorganic nitrogen compounds were tested for utilization by *F. effusum* in a modification of a basal semisynthetic medium described by Lilly and Barnett (12, p 427). Each substitution for asparagine in the medium was used at a rate providing an amount of nitrogen equivalent to that provided by 2g anhydrous asparagine (0.4242g N) (IX conc.). The compounds are listed in Tables 1 and 2.

During prior tests (unpublished), each constituent of the medium, except agar and trace elements, had been tested at several rates to determine optimum rates for maximum growth of *F. effusum*. The data were used to formulate a medium of the following composition: 20g dextrose, 2g KH_2PO_4, 1g $MgSO_4 \cdot 7H_2O$, 1 ml thiamine soln (100 mg/100ml), 2 ml of a trace elements solution (12, p 421), an inorganic nitrogen source at the desired rate, 15 or 20 g of agar and sufficient distilled water to bring the volume to one liter. The quantity of agar used was based on the pH desired. Twenty grams of agar were used for the acid series.

Early in this study it was found that some compounds made the medium so acid that the agar hydrolyzed during preliminary heating. Therefore, a preliminary pH adjustment was always made before melting the agar and a final adjustment was made prior to autoclaving. The following procedure was followed. All ingredients, except agar, were dissolved in approximately 950 ml of distilled water, the pH was adjusted with 2 M NaOH or HCl to a value about 0.7 higher than the desired pH (4.5 and 6.8). Agar was added, the volume was brought up to one liter and the medium was heated to melt the agar. The pH was again adjusted to a value 0.5 to 0.7 higher than desired. The

Table 1. Growth of *Fusicladium effusum* after 3 weeks on an agar-solidified, chemically-defined, acidic medium containing a single inorganic nitrogen compound.

Nitrogen compound[a]	ipH of medium (after sterilization)	Number colonies measured	Av. colony diameter (mm)[b]	Colony characteristics
$Zn(NO_3)_2 \cdot 6H_2O$	4.6	58	31	Dk. gray & flat
$Mn(NO_3)_2$	4.4	33	29	Med. gray & sl. mounded
$NaNO_3$	4.4	84	28	Dk. gray & flat
$Al(NO_3)_3 \cdot 9H_2O$	4.8	51	27	Brownish-gray & flat
$Mg(NO_3)_2 \cdot 6H_2O$	4.7	60	27	Dk. gray & flat
KNO_3	4.3	47	26	Dk. gray & flat
$Pb(NO_3)_2$	4.3	65	26	Dk. gray & flat
$Bi(NO_3)_3 \cdot 5H_2O$	4.5	71	25	Dk. gray & sl. mounded
$LiNO_3$	4.2	72	24	Dk. gray & sl. mounded
$Ce(NO_3)_3 \cdot 6H_2O$	4.7	73	24	Dk. gray & flat
None (Control)	4.3	87	23	Lt. gray & sl. mounded
$NaNH_4HPO_4 \cdot 4H_2O$	4.7	73	23	Med. gray & mounded
$Fe(NO_3)_3 \cdot 9H_2O$	4.2	80	22	Dk. gray & flat
NH_4NO_3	4.4	80	21	Lt. gray & mod. mounded
$Cu(NO_3)_2 \cdot 3H_2O$	4.5	84	21	Dk. gray & sl. mounded
$(NH_4)_2SO_4$	4.5	76	19	Lt. gray & mounded
$Ca(NO_3)_2 \cdot 4H_2O$	5.4	40	19	Whitish & flat
$(NH_4)_2HPO_4$	4.3	66	19	Greenish-gray & mounded
NH_4Cl	4.7	80	18	Dk. gray & flat
$(NH_4)_6Mo_7O_{24}$	4.7	95	14	Whitish & mounded
KNO_2	4.4	87	7	No growth
$NaNO_2$	5.1	62	7	No growth
$Co(NO_3)_2 \cdot 6H_2O$	4.4	63	7	No growth

[a]Amount used was the amount necessary to provide an amount of nitrogen equivalent to that furnished by 2g of anhydrous asparagine (0.4242g N).
[b]Diameter of inoculum discs = 7 mm.

medium was then dispensed into flasks and autoclaved for 20 minutes at 121°C. After sterilization, each batch of medium was poured into sterile petri dishes (Approx. 30 ml/dish). About 50 ml of each batch was saved for determination of initial final pH (ipH).

Cultures of *F. effusum*, originally derived from a single-spore isolate (Converse W-11), were used throughout this investigation. The original isolate was obtained from a foliar lesion on a Western cultivar pecan tree. Each dish of medium was "inoculated" with three 7 mm discs aseptically cut from the peripheries of 4-7 week old colonies on Proteose Peptone #2 (Difco)-dextrose agar (1). The discs were placed upside down in an equally-spaced triangular pattern. All dishes were held at 25C, the optimum growth temperature for the fungus (13).

Table 2. Growth of *Fusicladium effusum* after 3 weeks on an agar-solidified chemically-defined, near-neutral medium containing a single inorganic nitrogen compound.

Nitrogen Compound[a]	pH of medium (after sterilization)	Number colonies measured	Av. Colony diameter (mm)[b]	Colony characteristics
$Fe(NO_3)_3 \cdot 9H_2O$	6.1	80	25	Dk. gray & flat
KNO_3	6.9	9	24	Dk. gray & flat
$NaNO_3$	6.7	58	24	Dk. gray & flat
NH_4CL	6.2	81	24	Dk. gray & flat
None (Control)	6.8	78	23	Dk. gray & flat
$Pb(NO_3)_2$	6.7	63	23	Dk. gray & flat
$Ce(NO_3)_3 \cdot 6H_2O$	6.1	79	23	Dk. gray & flat
$Bi(NO_3)_3 \cdot 5H_2O$	6.8	91	22	Dk. gray & sl. mounded
$Zn(NO_3)_2 \cdot 6H_2O$	6.7	27	22	Dk. gray & flat
$Ca(NO_3)_2 \cdot 4H_2O$	6.4	36	21	Dk. gray & flat
NH_4NO_3	6.7	78	21	Whitish & mod. mounded
$(NH_4)_2SO_4$	6.7	45	21	Greenish-gray & flat
$Mg(NO_3)_2 \cdot 6H_2O$	6.7	42	20	Dk. gray & flat
$Mn(NO_3)_2$	6.9	56	21	Med. gray & flat
$Al(NO_3)_3 \cdot 9H_2O$	6.7	49	18	Brownish-gray & flat
$(NH_4)_2HPO_4$	6.8	73	17	Greenish-gray & flat
$Cu(NO_3)_2 \cdot 3H_2O$	6.2	95	14	Dk. gray & flat
$(NH_4)_6Mo_7O_{24}$	6.6	75	12	Dk. gray & flat
$NaNH_4HPO_4 \cdot 4H_2O$	7.1	45	11	Med. gray & mounded
$NaNO_2$	6.8	56	10	Lt. gray & flat
$Co(NO_3)_2 \cdot 6H_2O$	7.3	96	10	Lt. gray & flat
$LiNO_3$	6.8	75	10	Dk. gray & flat
KNO_2	6.6	26	9	Lt. gray & flat

[a]Amount used was the amount necessary to provide an amount of nitrogen equivalent to that furnished by 2g of anhydrous asparagine (0.4242 g N).
[b]Diameter of inoculum discs = 7 mm.

A growth period of 21 days was arbitrarily selected because this fungus grows very slowly. The rather large volume of medium in each dish prevented excessive drying and shrinking, and also prevented wide variations in growth (4, 5, 6). Colony diameter was used as a criterion of growth (4). Because of the usual uniform roundness of developing colonies, single diameter measurements were recorded at the end of each test. The average of two measurements was recorded for each slightly off-round colony. A few irregularly shaped colonies were formed but were not measured.

Liquid Medium Tests

Cochrane (7) is of the opinion that the agar culture method is "decidedly

not adequate for nutritional studies". Therefore, the same basal synthetic medium was used in a liquid medium study with the same substitutions for asparagine, as described earlier, plus two additional compounds. The pH of each medium was adjusted to 4.8±0.1. Fifty ml aliquots were dispensed in lots of ten 250 ml Erlenmyer flasks. The flasks of media were stoppered with DiSPo foam plastic closures (Scientific Products) and autoclaved at 121C for 20 minutes. Each flask was "inoculated" with one 7 mm disc of *F. effusum*, as described earlier, and incubated at 25C for 21 days. The mycelium was harvested onto tared filter papers, washed three times with distilled water, oven dried at 100C and the average dry weight per flask for each compound was determined.

Results

Agar Plate Tests

In the "acid series" nearly half of the compounds tested allowed good to excellent growth of *F. effusum* (Table 1). Like many other fungi, *F. effusum* utilized nitrate compounds better than ammonium compounds. At an acid pH, nitrate salts of zinc, manganese, sodium, aluminum, magnesium and potassium were preferentially used. Because $NaNO_3$ and KNO_3 do not form insoluble phosphates during autoclaving, and are utilized well, these compounds would appear to be ideal compounds for use in future synthetic media studies with *F. effusum*. A series of concentrations (1/2X, 1X, 2X) of each compound, at both pH levels, were tested to find an optimum growth concentration. With KNO_3, the 2X concentration provided the greatest growth (Figure 1), but the 1/2X rate was the better rate for $NaNO_3$ (Figure 2). With KNO_3, growth increased with concentration, but the reverse occurred with $NaNO_3$.

Compounds containing an ammonium ion were somewhat inhibitory to growth of *F. effusum* in an acid medium. $NaNO_2$, KNO_2 and $Co(NO_3)_2 \cdot 6H_2O$ were highly toxic at near-neutrality and lethal in an acid medium.

At near-neutrality, none of the compounds allowed as good growth as occurred in an acid pH and the order of utilization was very different from that of the acid series (Table 2). Most of the compounds were toxic to *F. effusum* at a near-neutral pH.

Some compounds caused color changes in the mycelium and some caused changes in colony morphology (Tables 1 and 2).

Liquid Medium Tests

The order of utilization of the test inorganic nitrogen compounds (Table 3) was greatly different from the order of utilization on the agar-solidified medium at the same acid pH level. KNO_3 and $NaNO_3$ were the better utilized compounds. More compounds were utilized in the liquid medium than in the

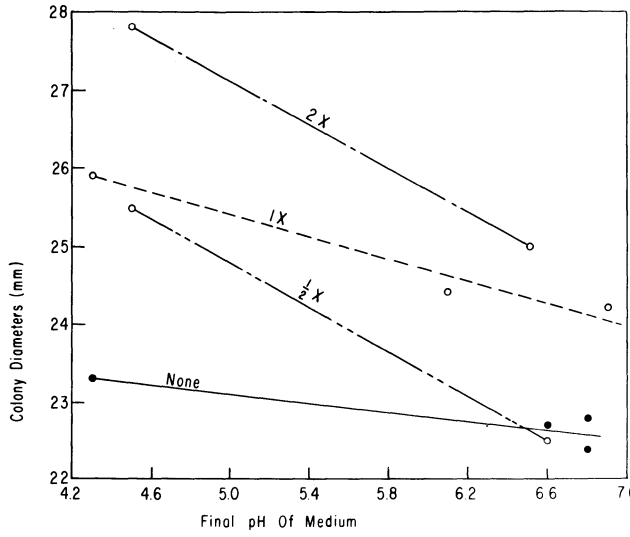

Fig. 1. Growth of *Fusicladium effusum* on an agar-solidified synthetic medium containing KNO_3 as the nitrogen source.

solid medium. Some compounds imparted characteristic colors to the medium and these colors were sometimes changed during autoclaving. Many times metabolic compounds imparted characteristic colors to the filtrates (Table 3). Nitrite compounds were very toxic in these tests as were the nitrate salts of copper and cobalt.

Discussion

Agar Plate tests

Like many fungi, *F. effusum* preferentially utilized nitrate compounds over ammonium compounds. The order of utilization at the two pH levels tested may be dependent on differential dissociation and permeation of the compounds at different pH values and physiological requirements for particular cations (6, 8, 9). The degree and order of utilization of nitrogen compounds may also vary with the carbohydrate source (9, p 492).

At an acid pH, the nitrate salts of zinc, manganese, sodium, aluminum, magnesium and potassium were preferentially used in the presence of dextrose, but at near-neutrality only the nitrate salts of iron, potassium, and sodium and NH_4Cl were preferentially used. The results with KNO_3 and $NaNO_3$ closely follow the results of Davis (8). All of the remaining compounds suppressed yields. The extreme toxicity of KNO_2 and $NaNO_2$ was not unex-

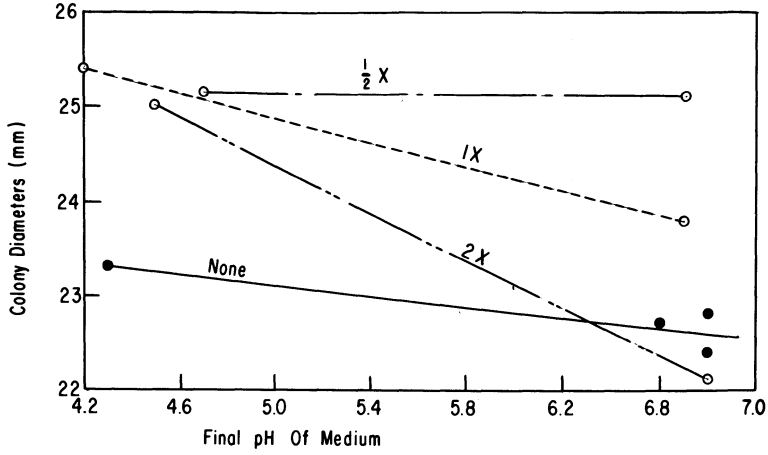

Fig. 2. Growth of *Fusicladium effusum* on an agar-solidified synthetic medium containing $NaNO_3$ as the nitrogen source.

pected. These compounds are very toxic to most fungi, especially in acidic media (7). HNO_2, which is formed in an acid medium, is very destructive to amino acids and proteins.

The toxicity of $Co(NO_3)_2 \cdot 6H_2O$ correlated with information reported by Cochrane (7) who stated that the cobalt ion, at appreciable concentration, is toxic to fungi and other organisms. The significance of changes in color and morphology of colonies of *F. effusum* induced by certain compounds is not known.

Liquid Medium Tests

There was less indication of preferential utilization of nitrate compounds over ammonium compounds than was shown in the agar plate tests. The top four better utilized compounds were nitrate compounds. Of these, KNO_3 and $NaNO_3$ were the better utilized compounds. Either one of these compounds could be used satisfactorily in future tests with chemically-defined media. KNO_3 would be the best choice since this compound would also provide readily utilized potassium ions. The toxicity of nitrite compounds and cobalt nitrate has been discussed earlier.

Summary

It is inferred from the data that utilization of inorganic nitrogen compounds by *F. effusum* follows the usual fungal pattern of preferential utilization

of nitrate compounds over ammonium compounds. *F. effusum* preferentially utilizes nitrate compounds, particularly in a very acid agar medium. The order of nitrate utilization apprently depends on the physiological requirements for particular cations and the pH of the medium. The order of utilization was different at each pH level possibly because of differential permeation and dissociation.

Many compounds, at the rates used, were inhibitory to *F. effusum*, particularly at near neutrality. $Co(NO_3)_2 \cdot 6H_2O$, KNO_2, and $NaNO_2$ were very toxic to this fungus. At near neutrality, $LiNO_3$ was also very toxic.

Some compounds cause color changes in the mycelium and some produce changes in colony morphology.

Table 3. Growth of *Fusicladium effusum* after 3 weeks on a chemically—defined, liquid medium (pH 4.8 ± 0.1) containing a single inorganic nitrogen compound.

Nitrogen compound[a]	Av. wt. of dried mycelium/flask (mg)	Filtrate	
		Final pH	Color
KNO_3	303.5	7.2	Blackish
$NaNO_3$	259.4	7.1	Blackish
$Mg(NO_3)_2 \cdot 6H_2O$	204.7	6.7	Clear
$Fe(NO_3)_3 \cdot 9H_2O$	195.0	6.9	Yellow
$Na(NH_4)HPO_4 \cdot 4H_2O$	175.7	2.5	Lt. tea
$(NH_4)_2HPO_4$	151.9	2.6	Blackish
$(NH_4)_2SO_4$	143.4	2.8	Clear
NH_4HO_3	119.2	2.6	Blackish
$Bi(NO_3)_3 \cdot 5H_2O$	102.7	5.8	Clear
NH_4Cl	96.9	2.5	Lt. tea
$Zn(NO_3)_2 \cdot 6H_2O$	86.8	4.3	Clear
$Ca(NO_3)_2 \cdot 4H_2O$	66.9	5.2	Clear
$Al(NO_3)_3 \cdot 9H_2O$	46.4	4.7	Clear
$Ce(NO_3)_3 \cdot 6H_2O$	38.4	4.6	Tea
$Mn(NO_3)_2$	37.4	3.8	Clear
None (Check)	36.7	4.9	Clear
$Ba(NO_3)_2$	30.2	4.2	Clear
$Pb(NO_3)_2$	21.3	3.4	Clear
$(NH_4)_6Mo_7O_{24}$	16.7	4.2	Dk. tea
$LiNO_3$	14.7	5.0	Clear
$ZnSO_4(NH_4)_2SO_4 \cdot 6H_2O$	7.8	4.2	Clear
$Co(NO_3)_2 \cdot 6H_2O$	7.4	2.8	Lt. orange
$Cu(NO_3)_2 \cdot 3H_2O$	6.6	4.1	Clear
KNO_2	1.0	6.4	Lt. yellow
$NaNO_2$	0.9	6.4	Lt. yellow

$NaNO_3$ and KNO_3 were considered to be the most satisfactory inorganic nitrogen compounds for use in synthetic media for growth of *F. effusum*.

This preliminary determination of efficient growth-promoting inorganic nitrogen sources has provided data useful for future investigations on carbohydrate, vitamin and trace element nutrition of this fungus.

Part II

Organic Nitrogen Sources

Nitrogen is an essential element that is used by fungi for functional as well as structural purposes. "Not all nitrogen sources are equally suitable for all fungi. Fungi may be specific in the nitrogen sources they utilize" (12). Utilization of nitrogen sources by the pecan scab fungus, *Fusicladium effusum*, has not been extensively investigated. Davis (8) has reported on results of tests with a very limited number and type of nitrogen compounds and a preliminary report by Barnes (2) on work on a wide variety of nitrogenous compounds has been published. The present report deals with utilization of many amino acids, amides and urea.

Materials and Methods

A modification of a chemically-defined medium described by Lilly and Barnett (12, p 427) was used in the study as a basal synthetic medium. The composition of the medium was as follows: 20g D-glucose, 2g KH_2PO_4, lg $MgSo_4 \cdot 7H_2O$, 1 ml thiamine soln (100 mg thiamine/liter), 2 ml of a trace elements soln (12, p 421) and sufficient distilled water to make one liter of medium. Each test nitrogen compound was individually included at a rate that provided an amount of nitrogen equivalent to that provided by 2g anhydrous asparagine/liter (0.4242 g N).

Almost all of the test compounds occur in proteins or as free compounds in biological systems. The few remaining compounds (L- ornithine, amino isobutyric acid, L-phenylserine, L-ethionine, and L-cysteic acid) are synthetic compounds with structures closely related to some of the naturally-occurring amino acids. Most of the compounds were obtained from Nutritional Biochemicals Corporation. The remainder were obtained from Calbiochem Corporation.

The above nutrient medium was prepared in such a way that no heat-induced reactions would occur between the amino acids and other ingredients of the medium (3). Each test amino acid, or related compounds, at an equivalent nitrogen weight was dissolved in individual aliquots of 900 ml of

distilled water. The large volume of water assured solubility of the least soluble test compounds. The pH was adjusted to 4.8 and 45 ml portions were dispensed into 250 ml Erlenmeyer flasks which were then closed with DiSPo foam plastic stoppers (Scientific Products) and autoclaved for 20 minutes at 121C. Amino acids are not hydrolyzed under these conditions.

The ingredients for each liter of nitrogen-free basal medium were dissolved in 50 ml of distilled water, the pH was adjusted to 4.8 ± 0.1 and the solution was sterilized with a sterile fritted glass filter to avoid heat-induced reactions. Five ml portions were then aseptically added to each cooled flask of autoclaved amino acid solution. Urea was included in these tests but it was filter sterilized to avoid degradation by heat. Each flask of complete medium was "inoculated" with a 7 mm disc of mycelium of *F. effusum* cut from the periphery of a 4-6 week old colony on a Proteose Peptone-#2 (Difco)-dextrose agar medium (1).

All cultures were incubated for 28 days in a 25C room lighted by fluorescent tubes for 9 to 12 hours daily. Mycelial mats were harvested on tared filter papers, washed with distilled water, oven-dried overnight at 100 C and weighed. The average weight per flask was calculated with each figure representing an average of 19 flasks. The pH of each culture filtrate was determined with a pH meter.

Results

Differential growth responses were obtained (Table 4). Of the 36 compounds tested, L-alanine provided the highest yields followed closely by L-norleucine, L-asparagine, L-creatinine, and L-glutamine. Those compounds not utilized were L-serine, L-methionine, L-tryptophan, L-sarcosine, L-canavanine, L-cysteine, L-cysteic acid, DL-dihydroxphenylalanine, and urea. None of the sulfur-containing amino acids tested, with the exception of L-djenkolic acid, was utilized. L-djenkolic acid was utilized to a very limited extent.

Discussion

Pelletier and Keitt (14) have stated that investigations with amino acids indicate that alanine, arginine, aspartic acid, glutamic acid, glycine and proline are "good" sources of nitrogen for fungi. Cochrane (7), after critically reviewing the literature on utilization of amino acids and amides, stated that "glycine, asparagine, glutamic acid, and aspartic acid are the most likely to support good growth". All of these authors agree that glycine, glutamic acid and aspartic acid are good amino acid sources of nitrogen for fungi in general.

Table 4. Utilization of certain amino acids, amides and urea by *Fusicladium effusum* in a chemically-defined medium.

Compounds	Initial pH (after autoclaving)	pH of filtrate	Av. yield/ flask (mg)
L-Alanine	5.2	5.1	120.4
L-Norleucine	4.8	4.5	101.5
L-Asparagine	4.8	6.5	96.1
L-Creatinine	4.8	5.0	86.1
L-Glutamine	4.8	6.0	80.5
DL-Amino n-butyric acid	4.8	5.2	77.9
L-Isoleucine	4.8	4.1	76.8
L-Valine	5.2	4.2	64.8
L-Aspartic acid	5.0	5.8	63.7
L-Hydroxy proline	4.8	4.2	60.4
L-Citrulline	4.8	4.2	50.4
L-Arginine	5.3	4.5	44.2
L-Glycine	4.8	5.1	43.7
L-Glutamic acid	4.8	5.2	42.9
L-Ornithine	5.3	3.8	38.5
L-Djenkolic acid	4.8	4.8	36.5
DL-Norvaline	4.8	5.0	36.0
L-Proline	5.4	5.3	34.9
L-Leucine	4.8	4.4	34.9
L-Phenylalanine	5.4	5.0	34.1
I-Amino isobutyric acid	4.8	6.2	33.5
L-Phenylserine	4.8	4.4	32.4
L-Threonine	5.3	5.2	24.7
L-Histidine	5.1	5.0	24.5
DL-Phenylalanine	4.8	4.9	15.7
L-Ethionine	4.0	3.9	15.6
None (Check)	4.8	4.3	15.0
L-Canavanine	4.8	3.8	9.9
L-Cysteic acid	4.8	2.5	8.9
DL-Dihydroxyphenylalanine	4.8	5.2	8.5
Urea	4.8	7.5	8.3
L-Sarcosine	4.8	2.4	8.1
L-Cysteine	5.4	4.2	7.5
L-Tryptophan	5.4	5.4	7.2
L-Serine	5.2	5.1	5.7
L-Methionine	4.9	5.0	5.3

Urea, another amide compound, is also generally recognized as a utilizable nitrogen source (7).

In a recent study on nitrogen nutrition of *F. effusum*, Davis (8) in a study with nine amino acids in Czapek solution agar, found that the fungus grew best with L-glycine and second best with DL-alanine, followed by L-histidine, L-arginine, L-lysine, L-tyrosine, L-glutamic acid, L-cystine and L-tryptophan. In the presently reported study, with a chemically-defined

liquid medium, *F. effusum* utilized L-alanine best followed by L-norleucine, L-asparagine, L-creatinine, L-glutamine, DL-amino n-butyric acid, etc. (see Table 1).

Davis did not test some of the higher-ranking compounds in this study so it is not known how *F. effusum* would have responded in his semi-synthetic medium. If Davis had used the L form of alanine rather than the DL form, alanine probably would have rated first in his study also, because fungi generally do not utilize the D form of amino acids. This phenomenon is demonstrated with phenylalanine in Table 1. Glycine would then have been rated second compared to a third place rating in the present study. When the same compounds used by Davis are compared in order of utilization in this study, a different order of utilization is apparent. The differences probably can be attributed to differences in fungus isolates and differences in type and constituents of media used.

It is interesting that L-tryptophan was not utilized in either study. Cochrane (7) and Pelletier and Keitt (14) have stated that this compound is poorly utilized by many fungi. *F. effusum* is no exception.

Of some significance is the lack of utilization, or very poor utilization, of the sulfur-containing compounds tested. It is known that many sulfur-containing compounds have fungitoxic properties (11). With some structural modifications of these molecules, it may be possible to synthesize new fungitoxic compounds.

The lack of utilization of urea by *F. effusum* would indicate that this fungus does not produce urease. Urease apparently is of general occurrence among fungi (7), but *F. effusum* is probably an exception.

It is impossible to compare utilization of amino acids and amides by various species in the genus *Fusicladium* because of the very limited number of compounds used by other investigators and the frequent use of the DL form rather than the L. form. The various workers have also used a variety of culture media.

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