



Studies on chemotaxis of *Aphanomyces cochlioides* Drech. zoospores to sugar beet seedlings
by Palthad Vittal Rai

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE in Botany (Plant Pathology)

Montana State University

© Copyright by Palthad Vittal Rai (1966)

Abstract:

Chromatographic analyses indicated that sugar beet root exudates contained 3 organic acids, 9 sugars and 14 amino acids. Quantitative analysis of these compounds showed that glucose, fructose, gluconic acid, maltose and xylose were in relatively large quantities.

Chemotaxis tests for zoospores of *A. cochlioides* with crude preparations of root exudates, individual fractions, the fractions in all possible combinations, and the individual compounds from neutral fraction and anion fractions showed that the crude preparations had the maximum attracting and growth influencing abilities, Gluconic acid had the maximum zoospore attracting ability among the individual compounds tested.

STUDIES ON CHEMOTAXIS OF APHANOMYCES COCHLIOIDES DRECH.
ZOOSPORES TO SUGAR BEET SEEDLINGS

by

Palthad Vittal Rai

A thesis submitted to the Graduate Faculty in partial
fulfillment of the requirements for the degree

of

MASTER OF SCIENCE

in

Botany (Plant Pathology)

Approved:

Richard H. M. Bell
Head, Major Department

Gay A. Strobel
Chairman, Examining Committee

K. Goering
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

June, 1966

10982

ACKNOWLEDGEMENTS

I take this opportunity to express my sincere gratitude to Dr. Gary A. Strobel for his advice and guidance throughout the course of this study. I acknowledge my indebtedness to Dr. M. M. Afanasiev; a word of his encouragement meant a continuation of my graduate studies.

Many thanks are extended to Dr. Gary A. Strobel, Dr. M. M. Afanasiev, Dr. E. L. Sharp, Dr. H. S. MacWithey and Dr. J. R. Welsh for their help in preparation of this manuscript.

TABLE OF CONTENTS

CHAPTER	PAGE
VITA	ii
ACKNOWLEDGEMENTS	iii
TABLE OF CONTENTS	iv - v
LIST OF TABLES	vi
LIST OF FIGURES	vii
ABSTRACT	viii
I INTRODUCTION	1
II MATERIALS AND METHODS	3
Culturing	
Zoospore suspension	
Sterile sugar beet root exudate	
Qualitative and quantitative analysis of root exudate	
Attraction tests and germination inducibility tests of:	
Crude preparation	
Anion fraction	
Neutral fraction	
Cation fraction	
Combination of the above fractions	
Individual compounds from the fractions	
III EXPERIMENTAL RESULTS	8
Compounds identified in:	
Anion fraction	
Neutral fraction	
Cation fraction	
Effect of the compounds on attraction and germination of zoospores	

CHAPTER	PAGE
IV DISCUSSION	24
V SUMMARY	27
VI LITERATURE CITED	28

LIST OF TABLES

TABLE		PAGE
I	Qualitative and Quantitative analysès of sugar beet root exudate	11
II	Role of sugar beet root exudate and its fractions in the phenomenon of attracting and influencing germination of <u>A. cochlioides</u> zoospores	12
III	Zoospore attraction towards individual compounds	13

LIST OF FIGURES

Figure	Page
1. Semidiagrammatic drawings of zoospore attraction, germination and development towards a crude preparation and fractions of a crude preparation of sugarbeet root exudate	15
2. Chromatogram of the organic acid fraction	17
3. Chromatogram of the neutral fraction	19
4. Thin layer chromatography of amino acid fraction	21
5. Attraction ratio of zoospores to different compounds	23

ABSTRACT

Chromatographic analyses indicated that sugar beet root exudates contained 3 organic acids, 9 sugars and 14 amino acids. Quantitative analysis of these compounds showed that glucose, fructose, gluconic acid, maltose and xylose were in relatively large quantities.

Chemotaxis tests for zoospores of A. cochlioides with crude preparations of root exudates, individual fractions, the fractions in all possible combinations, and the individual compounds from neutral fraction and anion fractions showed that the crude preparations had the maximum attracting and growth influencing abilities. Gluconic acid had the maximum zoospore attracting ability among the individual compounds tested.

CHAPTER I

INTRODUCTION

Afanasiev (1948) reported the occurrence of black root, or damping off of sugar beet (Beta vulgaris L.) caused by A. cochlioides, in Montana. Since then this pathogen has been observed to cause noticeable damage to the beet crop in Montana, especially in heavily irrigated soils. The symptomatology of this seedling disease is "black root", discoloration of hypocotyls varying from dark brown to black and discoloration of petioles of lower leaves. The leaves remain green and turgid, but diseased plants are stunted in growth (1).

Zoospores of A. cochlioides are the primary means by which this fungus asexually propagates. MacWithey observed massing of zoospores of A. cochlioides concentrated on the hypocotyl of sugar beet seedlings. He also observed that germination of zoospores was better when they clumped on the host (unpublished). Using Aphanomyces euteiches Cunningham and Hagedorn (3) reported that zoospores massed on pea roots, especially in the region of elongation. Dukes and Apple (4) discovered abundant massing of zoospores of Phytophthora parasitica var. nicotianae at the cut ends of roots and on the wounded parts. Zentmyer (16) showed that zoospores of Phytophthora cinnamomi were attracted to the excised roots of susceptible avocado plants. He also observed that the response of zoospores was more pronounced in the region of elongation than at the tip or in more mature portion of roots. Furthermore, he reported that germ tubes of these germinating zoospores were uniformly directed towards the root from a distance of up to 2-3 mm. He also demonstrated that the zoospores

of Phytophthora citrophthora were attracted to roots of its citrus host but not to those of avocado, indicating specific attraction of zoospores. Many other studies with different organisms have also shown that attraction of zoospores is general to root exudates (3, 4, 8, 15). Zoospores of A. cochlioides have been observed accumulating on sugar beet, pea (Pisum sativum) and tomato (Lycopersicum esculentum) (unpublished). Moreover, no accumulation was observed on cucumber (Cucumis sp.) roots.

Chemotaxis of zoospores has been worked out in various saprophytic fungi by many workers; and compounds such as potassium salts, inorganic phosphates and many protein degradation products, e.g., alanine, leucine, aspartic acid, glutamic acid, α -aminobutyric acid, etc., caused attraction (9, 10). Dukes and Apple (4) reported that 1% sucrose solution acts as a strong attractant of zoospores of Phytophthora parasitica var. nicotianae. They also observed that glucose, fructose, rhamnose, maltose and combinations of several sugars and amino acids attracted zoospores, but not lactose, galactose, tap water and sodium chloride. Carlile and Machlis (2) observed that zygotes of Allomyces sp. responded to individual amino acids, such as cystine, proline and serine. Royle and Hickman (9) reported that glutamic acid was unique in causing both attraction and encystment of zoospores of Pythium aphanidermatum. They also observed that combination of sugars, (fructose, glucose and sucrose) and 18 amino acids in equal proportions by weight caused excellent attraction and clustering of cysts. Troutman and Wills (15) stated that zoospores of Phytophthora parasitica var. nicotianae always migrated towards the

negative electrode in the presence of an electric current and compared this principle to that of plant roots and rhizosphere.

Although previous investigators have demonstrated the chemotactic properties of various compounds found in root exudates, no study had included the quantitative aspect of such compounds as they naturally occur. Furthermore, few investigators have even considered the complete qualitative analysis of compounds in exudates which act as attractants. It is therefore the purpose of this report to show which compounds are present in sugar beet exudate, what concentration of such compounds are exuded, and which compounds are effective in zoospore attraction, germination and development.

CHAPTER. II

MATERIALS AND METHODS

Preparation of zoospore suspension: An A. cochlioides culture (courtesy Dr. M. M. Afanasiev, Montana State University, Bozeman) was maintained on corn meal agar and grown on a liquid medium (5). The organism was grown in 250 ml Erlenmeyer flasks containing 100 ml of autoclaved medium for four days at room temperature. After decanting the medium, the mycelial mat was rinsed thoroughly in sterilized distilled water six times and incubated in the last rinse for 24 hours at room temperature. Spot tests indicated that sugars or amino acids were not present in the final rinse water. Twenty-four hours after the rinse, the mycelial mat produced an abundance of actively moving zoospores.

Preparation of sterile sugar beet root exudate: Sugar beet seeds of the Great Western Sugar Company, variety number 359-602, pretreated with New Improved Cerasan (0.3 g Cerasan per 100 g seeds) were treated with 20% Chlorox for 20 minutes. After washing the seeds 8 to 10 times in sterilized distilled water, they were aseptically transferred to plates of potato dextrose agar and incubated at room temperature for 3 days. The clean germinated seeds were transferred aseptically to the sterilized growth vessel.

The growth vessel was a petri plate (9 cm diameter and 4½ cm depth) containing stainless steel wire mesh fitted inside, 1 cm above water. The wire mesh acted as a platform on which the germinating seeds rested. The developing roots were held in the water in the vessel and shoots grew upwards from the platform. Hence the water in the vessel served as

a reservoir of root exudates. The seedlings were grown for seven days in the vessel at room temperature. The plants and exudates were checked for contamination on nutrient agar (Difco). The plants were counted and the water in the vessel was reduced to 1.0 ml by a flash evaporation.

Analysis of root exudates: The concentrated root exudate was passed through Dowex 50 (H^+) and Dowex 1 (formate), respectively, in order to separate the sample into cation, anion and neutral fractions, respectively. (13). The fractions were evaporated to dryness by dry air and placed in P_2O_5 , NaOH desiccator overnight.

The organic acid fraction was separated by one dimensional chromatography on Whatman No. 1 paper by using the following solvent systems: A) n-butanol - acetic acid - water (4:1:5 v/v), B) ethyl acetate - pyridine - water (8:2:1 v/v) and C) n-pentanol - 5 N formic acid (1:1 v/v). Organic acids were detected on the chromatograms according to the method of Trevelyan, et. al. (11), and by spraying of 5% brom-phenol blue in ethanol. Organic acids were quantitatively determined according to the method of Strobel and Hewitt (13).

Sugars were identified by one dimensional paper chromatography in solvent systems A and B. After elution from the chromatograms the reducing sugars were estimated quantitatively by the method of Nelson (6). Estimation of melibiose, raffinose and sucrose in the neutral fraction was made by Joyce-chromoscan densitometer, after treatment of the chromatogram with basic silver nitrate as prescribed by Trevelyan (14). Standard curves for these sugars were made by using 1, 2, 4, and 8 μg . Estimation

of sugars and organic acids were calculated on a per root basis.

A known amount of the amino acid fraction was separated by two-dimensional thin layer chromatography on silica gel H in the solvent system: Isopropanol-NH₄OH (67:33 v/v) followed by n-butanol-acetic acid-water (3:1:1 v/v). Known amino acids were also separated by two dimensional thin layer chromatography. Amino acids were detected by spraying 0.3% ethanol-ninhydrin on the developed chromatoplates. Amino acids present in the sample were identified according to their position corresponding to the position of the reference amino acids.

After separation of a given amount of sample the chromatoplates were air dried and sprayed twice with ethanolic ninhydrin and dried at 75 C for 10 minutes. The spots were scraped into a beaker with 7.65 ml distilled water, stirred well and filtered through Whatman No. 1 paper into a cuvette. Readings were taken in a Bausch and Lomb Spectronic 20 colorimeter at 570 m μ . Each reading was compared with the respective standard curve for that particular amino acid prepared in the same manner using known concentrations (0.5, 1.0, 1.5 and 2.0 μ g) of the amino acid. Individual amino acids were also calculated per root basis.

Attraction tests: To test root materials under standardized conditions, a modified technique of Royle and Hickman was used (8, 9). The capillary root model was prepared with glass capillary tubes of 1 mm outer diameter and 8 cm in length. Two scratches were made at the 2 cm mark in each tube. The tubes were washed thoroughly in concentrated sulfuric acid and sterilized distilled water. Solutions for tests were mixed in equal

proportions with 0.5% purified agar (Difco) at about 50 C. Capillaries were filled by allowing the agar solutions to be drawn up by capillary action to the 4 cm mark (20 μ l). After a few minutes when the substances inside the capillary tubes solidified, pieces of 2 cm length were made at the pre-cut marks. These root models were cleaned with cheesecloth and placed in plain Syracuse watch glass (diameter 2 5/8 inch) which was placed on the stage of a compound microscope. Two such root models were tested in each watch glass. There were 4 root models for each compound and the various fractions from beet root exudates. Agar, 0.25%, was used as a control in the root models.

Two ml of zoospore suspension were used in each watch glass. The tubes were arranged parallel to each other about 2 cm apart and the watch glass was covered with a lid. Readings were taken 6-8 hrs after the zoospore suspension was added. Concentrations of crude exudate and exudate fractions in the tubes were identical to the amounts produced by 70 plants. The concentration of other compounds in the tubes were identical to the amount produced by 5, 10, 15 and 20 plants. Readings were taken by counting the zoospores which lodged at the ends of root model in the microscope field. Furthermore, the germinating zoospores in each case were estimated. Readings of the randomly lodged zoospores were taken from randomly selected regions in the watch glass where there was no influence of the compounds which were in the root models. The proportion of zoospores at the root model ends to that of randomly lodging zoospores was calculated. The percentage of spore germination was also calculated in each case.

CHAPTER III

RESULTS

Gluconic acid was the predominant acid in the organic acid fraction (Table I, fig. 2). Two other compounds were present, lower in amount and detected in the solvent system, containing ethyl acetate-pyridine-water (8:2:1 v/v); the R_f 's of which were 0.28 and 0.56. The neutral fraction yielded 8 sugars, fructose, glucose, melibiose, raffinose, ribose, sucrose, and 1 unidentified compound (fig. 3). Table I shows that glucose was present in quantities larger than any other sugar. Fourteen spots were found on the chromatoplates when the amino acid fraction was analysed. Eight of these were identified; these include alanine, arginine, aspartic acid, glutamic acid, glycine, lysine, phenylalanine and threonine (fig. 4). The quantitative estimation of each compound is presented in Table I.

Zoospores showed distinct differences in response towards different fractions tested in root models as shown in Table II. The crude preparation had an excellent ability to attract zoospores to support a high germination and to influence profuse mycelial growth. The mycelial growth was more prominent near the tip of the root model than at the farther regions (fig 1-B). The amino acid fraction was next best to crude preparation in supporting the development of the germ tubes, but it did not have a noticeable ability to attract zoospores. The zoospores lodged near the end of the root model which contained amino acid fraction germinated and developed much better than the others which lodged farther away from the tube ends (fig. 1-C). The neutral fraction showed a relatively good zoospore attracting ability, however, it somewhat retarded the germination of zoospores. The development of germ tubes in

the presence of the neutral fraction was poor, and the accumulation of zoospores seemed to be diffuse (fig. 1-D). Second to the crude preparation, the organic acid fraction showed the maximum zoospore attracting ability (fig. 1-E); however, the organic acids appeared to have no effect on the germination of zoospores and the development of hyphae. When the amino acid fraction was combined with neutral fraction there was no zoospore attraction above that of the neutral fraction alone. Likewise, the zoospores germinated and developed as they did in the amino acid fraction alone. The combination of the amino acid fraction and the organic acid fraction showed a poor zoospore attracting ability when compared to organic acid fraction alone. When the total effect of this combination was compared with the individual effects, the amino acid fraction seemed to suppress the attraction ability of the organic acid fraction. However, there was slight increase in the attraction ratio and germination percentage over the results observed in the amino acid fraction alone. When the organic acid fraction and the neutral fraction were combined the attraction ratio was less than the individual effect of each fraction but the germination percentage was only slightly less than the additive effect of both the compounds. The combination of all the three fractions (fig. 1-F) had relatively a better effect on attraction, germination and development of the fungus but in all cases was less than that of crude preparation. The check (fig. 1-A) had an attraction ratio of 1, which was considered as the base and 10% germination.

The ability of the different compounds and groups of compounds to attract zoospores were made according to the following formula:

$$\text{Ratio of zoospore attraction} = \frac{\text{No. of zoospores at the end of root model}}{\text{No. of zoospores randomly lodging}}$$

Among the individual compounds gluconic acid showed maximum ability to attract zoospores with fructose and glucose next in order (Table III). Maltose, sucrose, and xylose played a relatively small role in attracting zoospores. Melibiose had no effect whereas raffinose and ribose seemed to repel the zoospores. Gluconic acid and all the identified sugars from the root exudate were tested for attraction of zoospores in root models using concentrations of compounds as they were found to be exuded by 5, 10, 15 and 20 plants, respectively, (fig. 5). When all of the identified sugars were combined with gluconic acid the attraction ratio was more than that of the sugars alone and less than that of gluconic acid alone. Amino acids were not tested individually as the amino acid fraction did not show attraction for zoospores under conditions as they naturally occurred in sugar beet root exudate.

TABLE I

Qualitative and quantitative analyses of sugar beet root exudate

Compounds exuded by sugar beet roots	Quantity in μg per root
I. ORGANIC ACIDS	
1. Gluconic acid	0.3560
II. SUGARS	
1. Fructose	0.4444
2. Glucose	1.1389
3. Maltose	0.2556
4. Melibiose	0.0063
5. Raffinose	0.0133
6. Ribose	0.0778
7. Sucrose	0.0002
8. Xylose	0.1556
III. AMINO ACIDS	
1. Alanine	trace*
2. Arginine	0.0024
3. Aspartic acid	0.0022
4. Glutamic acid	0.0021
5. Glycine	trace
6. Lysine	trace
7. Phenylalanine	trace
8. Threonine	trace

* Any compound which was less than 0.0001 μg was considered as trace.

TABLE II

Role of sugar beet root exudate and its fractions in the phenomena of attracting and influencing germination of zoospores of A. cochlioides

Fractions [*]	Ratio of zoospore ^{**} attraction	Germination percentage
Check	1.0	10
Crude preparation	5.5	82
Amino acid fraction	1.4	51
Neutral fraction	3.2	15
Organic acid fraction	3.9	12
Amino acid + neutral fraction	1.9	70
Amino acid + organic acid fractions	1.7	55
Organic acid + neutral fractions	3.1	23
Amino acid - organic acid - neutral fractions	3.6	54

*Fractions have been used from exudates obtained from 70 seedlings and the data given are the average of 4 replications.

**
$$\text{Ratio of zoospore attraction} = \frac{\text{No. of zoospores at the end of root model}}{\text{No. of zoospores randomly lodging}}$$

TABLE III

Zoospore attraction towards individual compounds

Compounds	Ratio of zoospore attraction				
	Quantity of compounds used (on a plant basis)				
	Ck	5	10	15	20
Gluconic acid	1.0	1.6	2.6	3.5	3.7
Fructose	1.0	1.2	1.5	1.7	2.2
Glucose	1.0	1.5	1.7	1.9	2.1
Maltose	1.1	1.4	1.5	1.5	1.5
Melibiose	1.0	1.0	1.0	1.0	-
Raffinose	1.0	1.1	-0.7	-0.7	-0.9
Ribose	0.9	1.2	1.0	0.9	0.8
Sucrose	1.0	0.9	1.1	1.2	-
Xylose	1.0	1.1	1.1	1.1	1.2

Fig. 1.

Semi-diagramatic drawings of zoospore attraction, germination and development of A. cochlioides towards a crude preparation and fractions of a crude preparation of sugar beet root exudate 8 hours after treatment. (The exudate was collected from 70, 7 day old sugar beet seedlings). The microscopic field examined at the root model tip was 100 X.

- A) Check
- B) Crude preparation
- C) Amino acid fraction
- D) Neutral fraction
- E) Organic acid fraction
- F) Amino acid fraction + neutral fraction + organic acid fraction

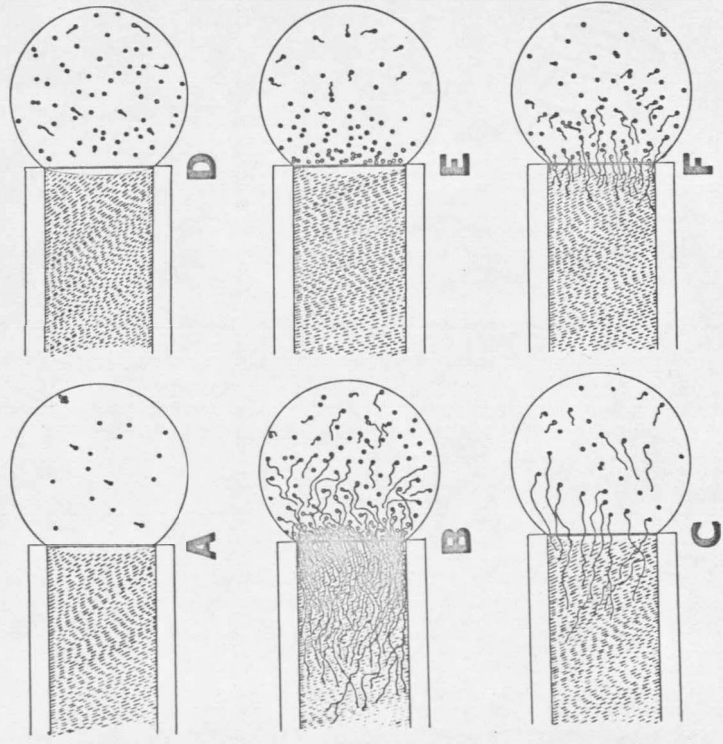


Fig. 2.

Chromatogram of the organic acid fraction from sugar beet root exudate, collected from 20 plants developed in a solvent system containing butanol : acetic acid : water (4:1:5 v/v) for 24 hours

1 and 2 - Different concentration of the sample

R - Reference

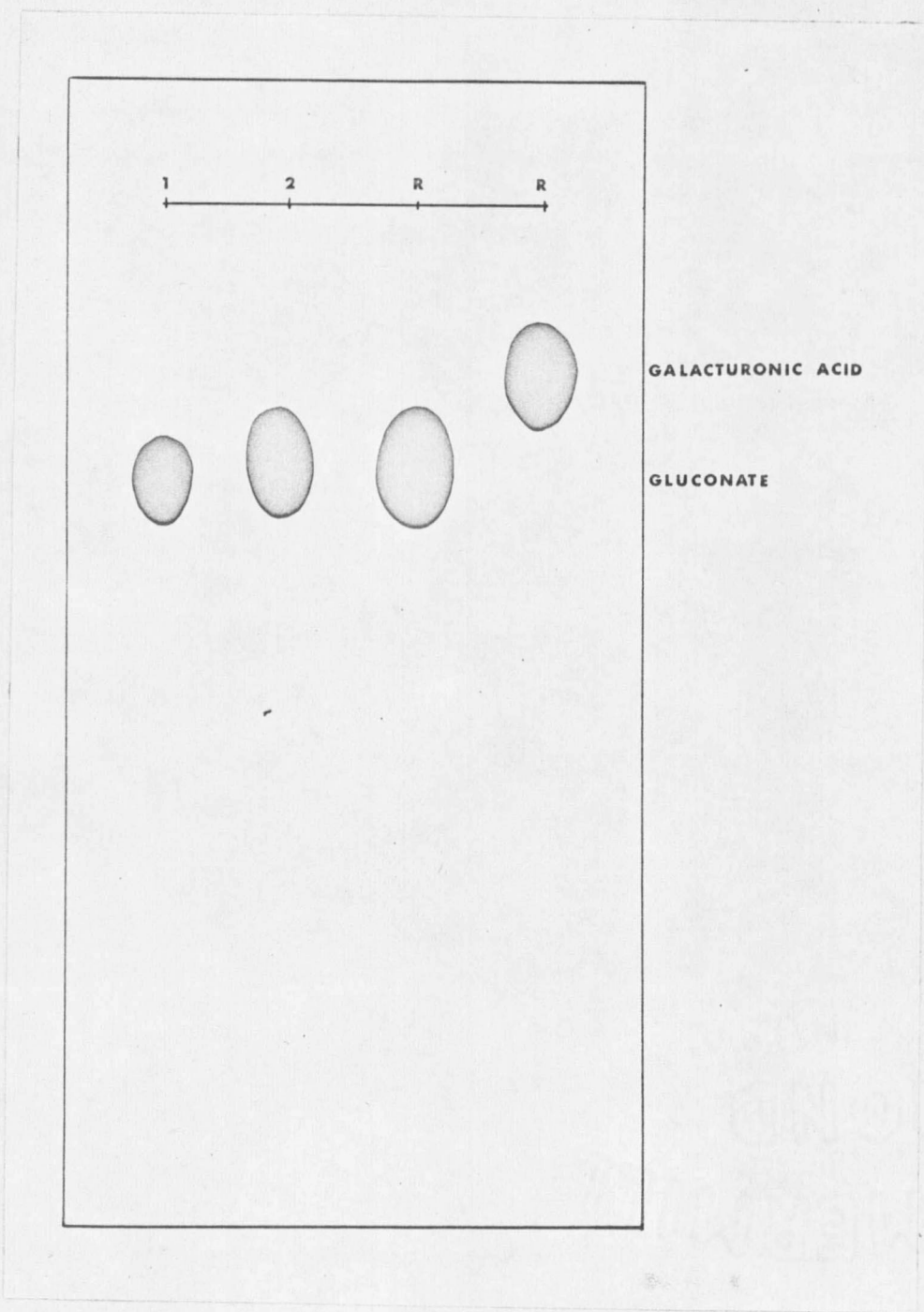


Fig. 3.

Chromatogram of the neutral fraction from sugar beet root exudate collected from 20 plants developed in a solvent system containing ethylacetate : pyridine : water (8:1:2 v/v) for 20 hours

R - Reference

S - Sample

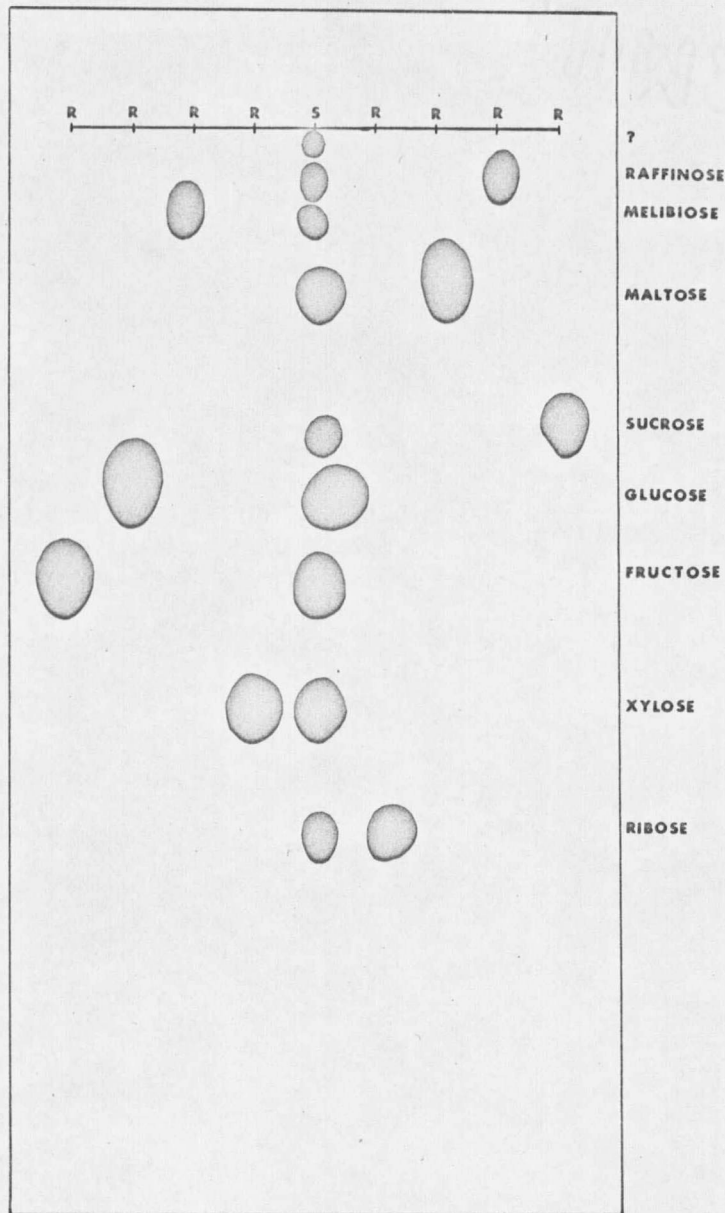
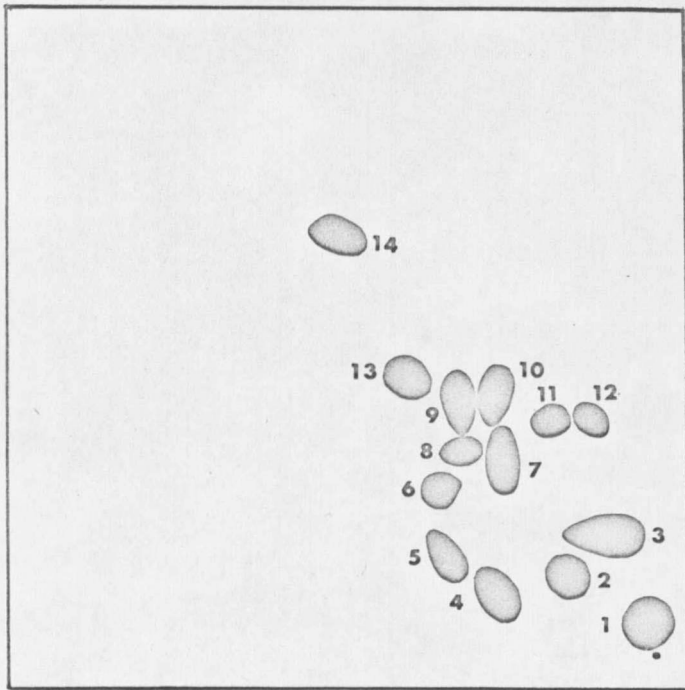


Fig. 4.

Thin layer chromatography of the amino acid fraction of sugar beet root exudate collected from 20 plants; developed first in a solvent system containing Isopropanol:NH₄OH, (67:33 v/v) and the next phase, butanol : acetic acid : water (3:1:1 v/v).

1) ? 2) arginine 3) lysine 4) aspartic acid 5) glutamic acid
6) ? 7) glycine 8) ? 9) alanine 10) threonine 11) ? 12) ?
13) ? 14) phenylalanine



↑
ISO PROPANOL
NH₄OH (67 : 33)

←
B A W (3:1:1)

Fig. 5.

Attraction ratio of zoospores to different compounds of sugar beet root exudate tested in a simulated condition

○———○ Gluconic acid

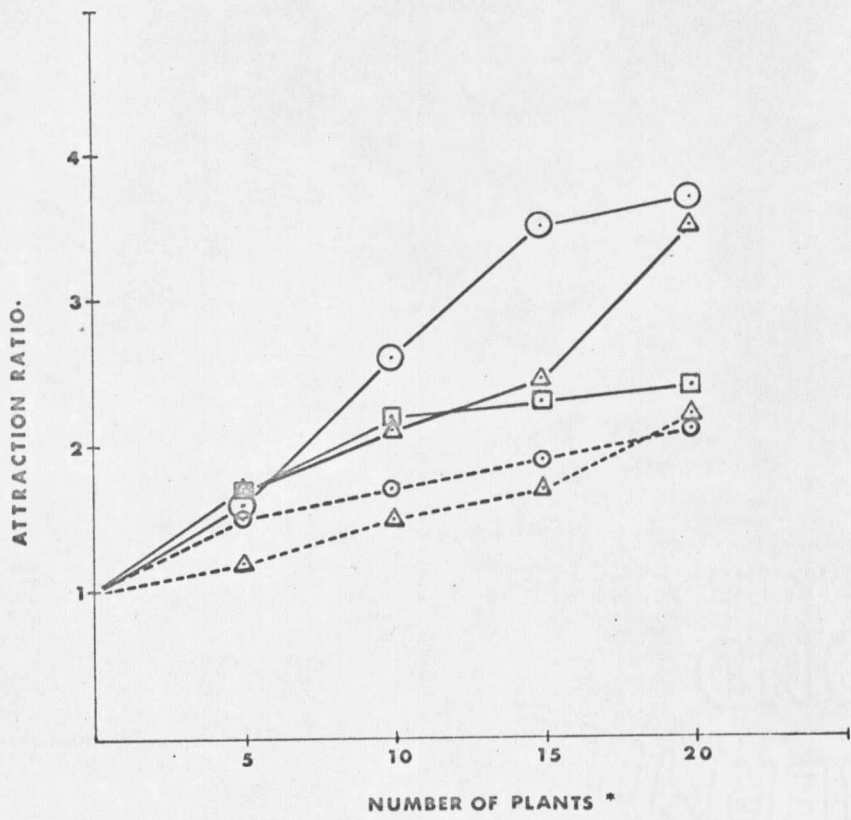
△———△ Gluconic acid + all the sugars which are identified from the root exudate

□———□ All sugars which are identified from the root exudate

○-----○ Glucose

△-----△ Fructose

* The concentration of different compounds were taken as they occur in 5, 10, 15, and 20 plants. (See Table I)



CHAPTER IV

DISCUSSION

Sugar beet root exudate contains at least 14 amino acids, three organic acids and 9 sugars. Rovira (7) reported that young pea root exudate contained 22 amino acids and 2 sugars which differed from that of oats which had 14 amino acids and 2 sugars. These results reveal that each plant may have a unique pattern of chemical exudation. This may be the reason why there is some specificity in the ability of certain plants to attract spores of different fungi.

The results indicate that the crude preparation has maximum zoospore attracting ability, the best effect on the stimulation of germination of zoospores, and subsequent growth and development of germ tubes. Thus, it appears that the crude preparation provides the fungus all the necessary factors for growth and development. On the other hand, the amino acid fraction showed a pronounced affect in promoting germination and development of the fungus but not in attracting the zoospores. The organic acid fraction showed the best ability to attract zoospores but did not seem to affect germination and growth of the fungus. The neutral fraction played a role in attracting the zoospores but appeared not to have an effect on germination, growth and development of the fungus. Thus, it seems as if each group of compounds has a particular function in the biological phenomenon of the A. cochlioides - sugar beet relationship.

When all the fractions were combined and tested for attraction and growth, the attraction ratio as well as the germination percentage and rate of growth were much less than that of the crude preparation (Table II). This might be explained on the basis that the crude preparation contained

biological factors which were destroyed during the experimental procedures.

The combination of organic acid and neutral fractions from the root exudate of 70 seedlings had a zoospore attraction ratio 3.1 (Table II) whereas the gluconate and sugars combined in concentrations simulating those of 20 plants was 3.4. Thus, the results of the tests are not strictly comparable. Several reasons may explain this discrepancy: 1) the relationships between attraction ratio and attractant concentrations are not linear, thus increased concentrations of attractants beyond that exuded by 20 plants would not proportionally increase the attraction ratio, 2) not all of the compounds in the exudate fractions were identified, thus, anions, other organic acids and neutral compounds not detected by the methods outlined may have a negative effect on zoospore attraction. This is illustrated by the repelling effect that higher concentration of raffinose has on zoospores (Table III).

Royle and Hickman (9) reported that glutamic acid and a combination of amino acid and sugar mixtures produced affects resembling those of pea root materials (crude preparation) in attraction and other functions such as encystment and development of Pythium aphanidermatum zoospores. The present study revealed that amino acids in concentrations simulating those from sugar beet root exudate did not have a role in attracting the zoospores of A. cochlioides but played a major role in supporting germination and development of the fungus.

Dukes and Apple (4) reported that 1% sucrose solution acted strongly to attract zoospores of Phytophthora parasitica var. nicotianae. However, in the present study glucose and fructose served as better attractants than sucrose under the simulated conditions of sugar concentrations in root exudate. Since sucrose is found in relatively minute quantity in the root exudate it may be ineffective in zoospore attraction.

Troutman and Wills (15) showed that zoospores of Phytophthora parasitica var. nicotianae always migrated towards the negative electrode and the plant roots exhibited a negative charge. Hence they stated that the attraction of zoospores towards the rhizosphere and roots is due to electrotaxis. Although no evidence of electrotaxis is presented in this report it is possible that it also occurs along with chemotaxis in attracting zoospores of A. cochlioides to sugar beet roots.

CHAPTER V

SUMMARY

Root exudates of sugar beet seedlings were separated into amino acid, neutral, and organic acid fractions by the appropriate Dowex exchange resins. Chromatographic analyses of the different fractions yielded 14 amino acids, 3 organic acids, and 9 sugars. Quantitative analyses of these compounds were done by standard techniques.

Chemotaxis tests for zoospores of A. cochlioides with crude preparations of root exudate, individual fractions, and the fractions in all possible combinations showed that crude preparations had the maximum ability to attract zoospores. The crude preparations also enhanced growth and development of the germ tubes. The amino acid fraction stimulated germination of the zoospores and growth and development of the germ tubes, but had no influence on attraction. The organic acid and neutral fractions had a high zoospore attracting ability but did not effect germination and development of the spores. Combination of the fractions revealed intermediate effects in most cases.

Gluconic acid had the maximum attracting ability among the individual compounds tested under concentrations simulating those exuded by beet roots. Other compounds such as glucose and fructose contributed highly to zoospore attraction. Maltose, sucrose, and xylose showed slight ability to attract zoospores. Melibiose had a neutral effect, whereas raffinose and ribose repelled the zoospores.

LITERATURE CITED

1. Afanasiev, M. M. 1948. The relation of six groups of fungi to seedling disease of sugar beets in Montana. *Phytopathology* 38: 205-212.
2. Carlile, M. J. and L. Machlis. 1965. A comparative study of the chemotaxis of the motile phase of Allomyces. *Amer. Jour. Bot.* 52: 484-486.
3. Cunningham, J. L. and D. J. Hagedorn. 1961. Attraction of Aphanomyces euteiches zoospores to pea and other plant roots. *Phytopathology* 51:616-618.
4. Dukes, P. D., and J. L. Apple. 1961. Chemotaxis of zoospores of Phytophthora parasitica var. nicotianae by plant roots and certain chemical solutions. *Phytopathology* 51:195-197.
5. MacWithey, H. S. 1960. In vitro inoculation of sugar beet seedlings with Aphanomyces cochlioides Drechs. *Amer. Soc. Sug. Beet Technologists.* 11:309-312.
6. Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.* 153: 375-380.
7. Rovira, A. D. 1956. Plant root excretions in relation to the rhizosphere effect. I. Nature of root exudate from oats and peas. *Plant Soil* 7:178-194.
8. Royle, D. J. and C. J. Hickman. 1964. Analysis of factors governing in vitro accumulation of zoospores of Pythium aphanidermatum on roots. I Behavior of zoospores. *Can. J. Microbiol.* 10:151-162.

9. Royle, D. J. and C. J. Hickman. 1964. Analysis of factors governing in vitro accumulation of zoospores of Pythium aphanidermatum on roots. II. Substances causing response. Can. J. Microbiol. 10:202-219.
10. Schroth, M. N. and D. C. Hildebrand. 1964. Influence of plant exudates on root infecting fungi. Ann. Rev. Phytopathology. 2:101-132.
11. Strobel, G. A. 1963. A xylanase system produced by Diplodia viticola. Phytopathology 53:592-596.
12. Strobel, G. A. and T. Kosuge. 1964. Metabolism of organic acids during rots of grape berries by Diplodia viticola. Phytopathology 54:242-243.
13. Strobel, G. A. and Wm. B. Hewitt. 1964. Time of infection and latency of Diplodia viticola in Vitis vinifera var. Thompson seedless. Phytopathology 54: 636-639.
14. Trevelyan, W. E., D. P. Procter and J. S. Harrison. 1950. Detection of sugars on paper chromatograms. Nature 166:444-445.
15. Troutman, J. L. and W. H. Wills. 1964. Electrotaxis of Phytophthora parasitica zoospores and its possible role in infection of tobacco by the fungus. Phytopathology 54:225-228.
16. Zentmyer, G. A. 1961. Chemotaxis of zoospores for root exudates. Science 133:1595-1596.

