GROWTH OF PHYTOPHTHORA INFESTANS RACE 1.2.4 AND SYNTHESIS OF STEROID GLYCOALKALOIDS BY THE FUNGUS IN SYNTHETIC MEDIA

by

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ABSTRACT

Growth of Phytophthora infestans Race 1.2.4 and Synthesis of Steroid Glycoalkaloids by the Fungus in Synthetic Media

by

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A chemically defined synthetic medium was developed in which Phytophthora infestans (Mont.) de Bary race 1.2.4 grew extensively. This medium consisted of: 90.0 grams maltose, 20.0 grams DL-alanine, 20.0 grams L-proline, 1.0 gram KNO₃, 0.5 gram KH₂PO₄, 0.25 gram MgSO₄·7H₂O, 1.0 milligram thiamin per liter of distilled water. The average level of growth in this medium was 0.5864 gram dry weight mycelium per 50 milliliters medium.

The glycoalkaloids, solanidine and solanine, were synthesized by Phytophthora infestans in the above medium with 62.5 percent of the samples positive and an overall average of 0.85 milligram total glycoalkaloids per 25 milliliters medium. The level of alkaloids detected in the medium ranged from 0.0 to 5.9 milligrams per 25 milliliters medium. No glycoalkaloid production was detected when the level of growth of the fungus was below 0.35 gram dry weight mycelium per 50 milliliters medium.
Phytophthora infestans was also cultured on the following semi-synthetic media: chick pea medium, rye seed medium, and lima bean medium. The fungus grew extensively in each medium, but produced alkaloids in only the chick pea medium. Fifty percent of the samples of this medium were positive for alkaloids with an overall average of 0.67 milligram total glycoalkaloids per 25 grams chick peas.

Sodium salts of the macronutrients were individually substituted for the potassium and magnesium salts of the basal medium and sodium chloride and sodium acetate were singly added to the medium. Maximum growth levels were demonstrated when 0.04 percent phosphate or 0.04 percent chloride was employed in the medium. Sodium acetate was inhibitory to Phytophthora infestans.

Glycoalkaloid production was significantly reduced in media containing sodium salts of the macronutrients. Regression analysis showed that the concentration of phosphate in the medium had the most influence on the level of glycoalkaloids synthesized by the fungus. Raising the concentration of phosphate in the medium resulted in increasing amounts of alkaloids being produced by Phytophthora infestans.

Upon addition of micronutrients to the enriched basal medium, growth of Phytophthora infestans was stimulated by levels of ferrous iron in the concentration range of 1.0-5.0 milligram 1⁻¹. Phytophthora infestans was inhibited by all concentrations of cobalt, copper, manganese, nickel, and by concentrations of calcium greater than 0.001 milligram 1⁻¹.

No glycoalkaloid production by Phytophthora infestans was detected in media containing cobalt, copper, calcium, nickel, or
manganese. Glycoalkaloids were produced in media containing iron at all concentrations except 5.0 milligrams l\(^{-1}\) with the highest amount of alkaloids occurring in media containing 0.001 milligram l\(^{-1}\). Ferrous iron seems to inhibit glycoalkaloid synthesis by *Phytophthora infestans*. As the level of iron in the media was increased the quantity of glycoalkaloids produced in the media declined.

Studies were carried out to determine the effect of mineral nutrition on the resistance of potato foliage to infection by *Phytophthora infestans*. Results indicated that the level of phosphate had little or no effect on the susceptibility of potato leaflets to late potato blight. Levels of nitrate (1.0-10.0 percent) had little effect on the blight resistance of foliage, whereas higher levels of nitrate (20.0-25.0 percent) significantly increased the susceptibility of leaflets to infection by *Phytophthora infestans*.

Growth of common saprophytic and enteric bacteria was not inhibited by glycoalkaloids in the concentration range of 1 to 200 milligrams total glycoalkaloids per 100 milliliters.
INTRODUCTION

The potato (*Solanum tuberosum* L.) is an important commodity and has been fundamental in the economic history of the world. In 1976 the total yield of potatoes was nearly 300 million tons, with the Union of Soviet Socialist Republics, Poland, Germany, France, and the United States of America leading producers of the crop (Food and Agriculture Organization of the United Nations, 1977). Potatoes have become a staple in the diet of people in most areas of the world since they grow in a variety of soils and climates, yield heavily, and are relatively inexpensive to purchase. *Phytophthora infestans* (Mont.) de Bary, causative agent of the late potato blight, is and has been a significant factor in decreasing the yield of potatoes.

The potato originated in South America and was introduced into Europe in the 1500's; about 200 years later the potato was brought to North America. Historical records indicate that late potato blight first appeared in Europe and the United States around 1850. On August 23, 1845, "The Gardener's Chronicle and Agricultural Gazette" under the editorship of Dr. John Lindley published the first report on the appearance of late potato blight with the following words.

A fatal malady has broken out amongst the potato crop. On all sides we hear of destruction. In Belgium the fields are said to have been completely desolate. There is hardly a sound sample in Covent Garden Market. (Large, 1940, p. 24)

This new disease struck down healthy plants and quickly spread throughout Europe. During the following weeks reports of the disease were received from Poland, Germany, Belgium, France, and England.
On September 13, 1845, the presence of the potato murrain (blight) was reported in Ireland. In England failure of the potato crop was gaunt enough with even the poorest laborer in England living on oat gruel and bread, as well as potatoes. But in Ireland the paupers were living exclusively on potatoes. The late potato blight struck with a devastating force. The desolation in Ireland is illustrated by the passage:

On July 27, wrote Father Matthew, I passed from Cork to Dublin and the doomed plant bloomed in all luxuriance of an abundant harvest. Returning on August 3rd I beheld with sorrow one wide waste of putrefying vegetation. In many places wretched people were seated on the fences of their decaying gardens and wailing bitterly at the destruction which had left them foodless. (Large, 1940, p. 36)

As a consequence of the late potato blight epidemic in Ireland a million people died and one and one-half million emigrated (Large, 1940). During this period in Europe the late potato blight fungus revealed itself to be a new and formidable enemy of mankind. The destruction of the staple food supply of human society brought about more death and suffering since the Napoleonic Wars. The late potato blight epidemic in Ireland provided scientists with additional information concerning the nature of disease and brought about a new branch of applied science, plant pathology. At the start of the blight epidemic in Europe its specific cause was unknown. A British naturalist, Reverend M. J. Berkeley, connected the disease with a mold in the infected tissues (Large, 1940). Dr. Montagne of France described the fungus and named it Botrytis infestans (Large, 1940). In 1876 de Bary examined the morphology of the fungus and established a new genus for it, which he called Phytophthora. Figure 1 shows the fungus and the infected leaf. Phytophthora infestans is a member of the
Figure 1. Morphology of *Phytophthora infestans*.
Potato leaf section with *Phytophthora infestans* growing amongst the loose tissue of the underside of the leaf. 145X

Underside of the potato leaf with *Phytophthora infestans* growing out of the stomata. 100X
"Phycomycetes" and has a marked resemblance to *Pythium* and *Saprolegnia*, water fungi.

Control of the late potato blight is an ongoing problem. Meteorological conditions play a major role in the outbreak of late potato blight. The precise weather conditions necessary for the occurrence of late potato blight are: (1) night temperature not below 10°C; (2) night temperature below the dew point for at least 4 hours; (3) mean cloudiness not below 0.8 on the following day; and (4) rainfall of at least 0.1 mm on the following day (Walker, 1957). Since favorable weather conditions for the occurrence of a blight epidemic are defined, areas where late potato blight is a problem have established forecasting programs to predict blight epidemics based on meteorological conditions. During periods of optimal weather conditions growers can be warned and appropriate control measures can be taken.

In 1891 the first successful remedy for the late potato blight was developed. Control consisted of spraying foliage several times a week with a chemical mixture called the Bordeaux mixture, which consisted of 20 pounds of copper sulfate and 10 pounds of quicklime in 100 gallons of water (Large, 1940). This was applied in the concentration of 30 gallons per acre. At this time protective spraying was the only reliable method of controlling late potato blight.

Today blight is best controlled by utilizing organic fungicides such as Maneb, Zineb, and Daconil. During periods of blight infestations sprays of fixed copper, such as the Bordeaux mixture, are still recommended.
The macro- and micronutrients play a role in decreasing or increasing the susceptibility of plants to infections with plant pathogens. Some researchers have attempted to control late potato blight by utilizing mineral nutrition to increase plant resistance to the fungus. This method of control is centered in Europe and has shown varied results.

Steroid glycoalkaloids are bitter tasting compounds possessing toxic characteristics which are present in normal Irish potatoes in trace amounts. The major glycoalkaloids in the potato are derived from a steroid base, solanidine, which contains an alcohol hydroxyl group, a reducible double bond, a tertiary nitrogen with no methyl group, and four C-methyl groups with an empirical formula of \( \text{C}_{27}\text{H}_{43}\text{ON} \) (Schreiber, 1968). The principal glycoalkaloids, solanine and chaconine, are a combination of solanidine, a steroid base, and several carbohydrates. Solanine contains 1 mole of glucose, 1 mole of rhamnose, and 1 mole of galactose, whereas chaconine contains 1 mole of glucose and 2 moles of rhamnose (Figure 2).

Toxicity of these substances has been demonstrated in man, animals, and microorganisms. Cases of potato poisoning in man have been reported with gastrointestinal and neurological disturbances, and sometimes death. Outbreaks of poisoning have been reported in the United States since the turn of the century. One such case is narrated here.

About October 15, 1924, James B. Matheney, of Vandalia, Illinois, gathered about one and one-half bushels of tubers from their patch of strawed potatoes. The potatoes were green. . . . On October 18, the family started to use the greened potatoes and two days later began to show signs of poisoning. All members of the family, consisting
Figure 2. Structural formulas of solanine and chaconine.
of the father, mother, two daughters, and five sons were ill with exception of the father, who did not partake of the tubers, and a child of 18 months who lived almost exclusively on milk. The mother, age 45, died on October 25, and a daughter, Cynthia, age 16, died two days later. The other five members of the family recovered. (Hansen, 1925, p. 341)

Losses of livestock and poultry have been caused by ingestion of potato vines, sprouted potatoes, cull potatoes, and potato peels, all of which are high in glycoalkaloids (Hansen, 1925; Willimott, 1933). These materials have been exposed to light when discarded by factories or left in the field by farmers. Upon exposure to light, the biochemical pathways responsible for the synthesis of glycoalkaloids are activated, thus these substances contain an increased concentration of glycoalkaloids. Also, potatoes that have undergone "greening," i.e., chlorophyll has formed within the surface layers, have an elevated level of glycoalkaloids due to light induction of the biosynthetic pathway (Hilton, 1951).

The action of steroid glycoalkaloids as phytoalexins received minimal attention until the 1960's. In 1968 several investigators reported that *Fusarium solani* and *Helminthosporium carbonium* were inhibited by steroid glycoalkaloids which were detected in the potato.

Increased quantities of steroid glycoalkaloids are present in potato tubers which have been subjected to physiological stress. Stress conditions can be induced by environmental conditions, such as exposure to light, high relative humidity, and high storage temperatures. Mechanical damage incurred during harvesting, transporting, and processing also increases the physiological stress on tubers. Potatoes which have been infected with *P. infestans* also produce increased levels of glycoalkaloids.
Currently, emphasis has been placed on the study of *P. infestans* and glycoalkaloids due to a report by Renwick (1972) which implicated "blighted" potatoes containing unknown substances with the induction of birth defects in humans.

To study the growth of *Phytophthora infestans*, production of glycoalkaloids by the fungus, the effects of macronutrients on the resistance of potato foliage to *P. infestans*, and the toxicity of glycoalkaloids to bacteria, the following investigations were undertaken:

1. Development of a defined synthetic medium for culturing of *Phytophthora infestans* and production of steroid glycoalkaloids by the fungus.

2. Growth of *P. infestans* and synthesis of glycoalkaloids by the fungus in medium containing various concentrations of macronutrients.

3. Growth of *P. infestans* and production of glycoalkaloids by the fungus in medium containing micronutrients.

4. Control of infection of potato foliage by *P. infestans* through application of macronutrients to the soil.

5. Assessment of the toxicity of a natural mixture of glycoalkaloids to bacteria.
REVIEW OF LITERATURE

Literature pertaining to culturing of *Phytophthora infestans*, nutritional control of late potato blight, biochemistry, and toxicology of glycoalkaloids is summarized here.

Culturing of *Phytophthora infestans*

*Phytophthora infestans* is an ecological obligate parasite. This fungus has a low ability to grow saprophytically under natural and artificial conditions. For the most part, investigators have used natural or semisynthetic media to culture *P. infestans*. In 1953 French developed a defined medium containing glucose as the carbon source which he utilized to distinguish several physiological races of *P. infestans*. Other investigators have employed French's medium to culture isolates of *P. infestans* (Hall, 1959; Seidal, 1961). Seidal (1961) cultured *P. infestans* in a variety of sugars and found that the highest level of growth was obtained in medium containing glucose or sucrose. Some researchers have enriched French's medium by adding amino acids, such as aspartic acid (Thurston et al., 1959), asparagine (Hodgson, 1958), and glutamic acid (Fehrmann, 1971).

Some study of the metal ions required by *P. infestans* has been done. A number of investigators reported the addition of no trace elements to their media (French, 1953; Thurston et al., 1959; Hall, 1959). Others added iron but no other microelements (Hodgson, 1958; Seidal, 1961; Fehrmann, 1971). Cuppert and Lilly (1973) found that in a synthetic medium containing glucose and asparagine the addition
of 1.0 mg \(1^{-1}\) ferric iron with 200 mg \(1^{-1}\) L-ascorbic acid stimulated growth of \(P.\) \textit{infestans}. In this medium L-ascorbic acid reduces the ferric ion to the ferrous state which is readily utilized by the fungus. Frothergall and Child (1964) reported a 50\% increase in the growth of \(P.\) \textit{infestans} following addition of calcium to the basal medium.

\textit{Phytophthora infestans} has been found to synthesize complex organic chemicals in synthetic medium. In French's medium fortified with L-tyrosine and L-phenylalanine \(P.\) \textit{infestans} synthesized umbelliferone, heriarin, and coumarin (Austin and Clarke, 1966). They hypothesized that production of coumarins by \(P.\) \textit{infestans} was due to stress conditions imposed on the fungus because of growth in a synthetic medium. No reports have been observed on the production of glycoalkaloids by \(P.\) \textit{infestans} in a synthetic medium.

**Nutritional control of late potato blight**

Studies of the relationship between nutrition and the incidence of foliage blight have received much attention. Langbein and Pehl (1962) indicated that application of nitrogen to the soil increased the nitrogen content of the leaves and lowered the susceptibility of the plant to \(P.\) \textit{infestans}, but high levels of nitrogen induced leaf formation and facilitated the attack of \(P.\) \textit{infestans}. These data are supported by Weindlmayr (1965) who observed that high nitrogen levels increased the susceptibility of foliage to blight. The incidence of blight in tubers is also heightened with increasing nitrogen levels (Herlihy, 1970).
Application of phosphorus fertilizers appears to decrease the susceptibility of foliage and tubers to late potato blight (Weindl-mayr, 1965; Herlihy, 1970). Effectiveness of other macronutrients on increasing the resistance of potatoes to late potato blight has been studied. The use of sulfate enhanced the resistance of potato leaflets to infection by late potato blight (Borys, 1964). When elemental sulfur is mixed with the soil the incidence of blight may or may not decrease. Barnes and Chestnut (1966) found that utilization of sulfur reduced the incidence of blight, whereas Hutchinson and Mulligan (1971) reported that upon mixing sulfur with the soil the pH of the soil decreased but there was no significant reduction on the proportion of blighted tubers. Utilization of low levels of potassium (25-100 mg l\(^{-1}\)) decreased leaf resistance to \textit{P. infestans}, whereas higher levels (400 mg l\(^{-1}\)) increased resistance (Szczotka et al., 1973). Higher levels of potassium were also shown to increase lesion size and sporulation of the fungus (Umaerus, 1969).

Microelements have been demonstrated to play an important role in the resistance of potatoes to late potato blight. When copper, zinc, manganese, or molybdenum were bound to superphosphate, the susceptibility of foliage and tubers to late potato blight decreased (Mudich, 1967). Molybdenum at the 0.002-0.004\% level was the most effective, followed by manganese at the 0.1-0.2\% level. Studies by Isaeva (1969) indicated that application of nickel or cobalt significantly increased the resistance to blight. Use of copper or boron demonstrated a decrease of 20-50\% in the proportion of potato tubers infected by \textit{P. infestans} (Petrova, 1963).
Biochemistry of glycoalkaloids

The initial tracer work on the biosynthesis of potato glycoalkaloids was undertaken by Guseva and Paseshnichenko (1958). They demonstrated the uptake and incorporation of radioactive acetate by potato sprouts into solanidine, the aglycone, under illuminated conditions and into the sugar moiety of glycoalkaloids when sprouts were grown in the dark. Further experiments indicated that mevalonate was more efficiently utilized in the biosynthesis of potato glycoalkaloids by seedlings than acetate (Guseva, Paseshnichenko, and Borikhena, 1961). Cholestrol was shown to be metabolized to solanidine when applied to the leaf surface of potato plants (Tschesche and Hulpke, 1967).

Biosynthetically all steroidal compounds, such as sterols, terpenes, hormones, and alkaloids are interrelated and pathways leading to synthesis of structurally similar compounds could be postulated on the basis of known ones. Thus the pathways starting from acetate via mevalonate, isopentyl pyrophosphate, farnesyl pyrophosphate, squalene, and cholesterol is applicable to steroid glycoalkaloid synthesis (Heftmann and Mosettig, 1960). Figure 3 illustrates the synthetic pathway of steroid glycoalkaloids. The source of the nitrogen atom of glycoalkaloids is unknown, although according to Heftmann's (1967) hypothesis, cholesterol may undergo a cyclization in the side chain after the formation of 27-hydroxylcholesterol followed by a direct replacement of the hydroxyl group by an amine group.
Figure 3. Outline of biosynthesis of steroid glycoalkaloids.
ACETIC ACID (C2)

CH₂̅-COOH

MEVALONIC ACID (C8)

H

CH₂-OHP

FARNESYL PYROPHOSPHATE (C₁₅)

Δ₃-ISOPENTENYL PYROPHOSPHATE (C₅)

SQUALENE (C₃₀)

CHOLESTEROL (C₂₇)

GLYCOALKALOIDS (C₄₅)

R = SUGARS

SOLANIDINE (C₂₇)
Toxicology of glycoalkaloids

Incidents of poisoning of humans and animals due to the consumption of potato tubers are well documented (Harris and Cockburn, 1918; Rothe, 1918; Bomer and Mattis, 1923; Griebel, 1923; Hansen, 1925; Damon, 1928; Willimott, 1933; Wilson, 1959). In many cases the toxicity of the tubers was traced to high solanine content. Attempts at establishing the toxicity of solanine have been ongoing since 1895. Anticholinesterase activity of solanine has been demonstrated, but the toxic effects of this alkaloid have not been attributable to its inhibitory effect on cholinesterase (Pokrovskii, 1956; Orgell et al., 1958; Harris and Whittaker, 1959, 1962; Orgell, 1963; Patil, 1972). Through studies with tritiated solanine in rats, the metabolic fate and distribution of solanine was determined (Nishie et al., 1971). Their results indicated (1) partial as well as complete gastrointestinal hydrolysis, (2) poor absorption from the gastrointestinal tract, (3) rapid urinary and fecal excretion, and (4) build up of high but descending order of concentrations in various tissues including the spleen, kidney, liver, lung, fat, heart, brain, and blood.

Several animals have been tested for their sensitivity to solanine as well as total glycoalkaloids. These data are presented in Table 1. Human experiments indicated that an oral dose of 200 mg (about 2.8 mg Kg\(^{-1}\)) of solanine caused hyperesthesia, drowsiness, itchiness in the neck region, and dyspnea with higher doses causing vomiting and diarrhea (Ruhl, 1951).
Table 1. Evaluation of solanine toxicity

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Administration</th>
<th>Dose of solanine Amount</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human(^a)</td>
<td>Oral</td>
<td>2.8 mg/kg(^b)</td>
<td>Toxic(^c)</td>
<td>Ruhl (1951)</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>20-25 mg (b)</td>
<td>Toxic(^c)</td>
<td>Wilson (1959)</td>
</tr>
<tr>
<td>Sheep</td>
<td>Oral</td>
<td>225 mg/kg</td>
<td>Toxic(^c)</td>
<td>König (1953)</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>500 mg/kg</td>
<td>Lethal</td>
<td>König (1953)</td>
</tr>
<tr>
<td></td>
<td>Intravenous</td>
<td>17 mg/kg</td>
<td>Toxic(^c)</td>
<td>König (1953)</td>
</tr>
<tr>
<td></td>
<td>Intravenous</td>
<td>50 mg/kg</td>
<td>Lethal</td>
<td>König (1953)</td>
</tr>
<tr>
<td>Pregnant rat</td>
<td>Oral</td>
<td>10% sprout diet</td>
<td>Death of all pups before weaning age</td>
<td>Kline et al. (1961)</td>
</tr>
<tr>
<td>Rat</td>
<td>Gastric (\text{intubation})</td>
<td>590 mg/kg</td>
<td>50% death within 24 hours</td>
<td>Gull, Isenberg, and Bryan (1970)</td>
</tr>
<tr>
<td></td>
<td>Intraperitoneal</td>
<td>75 mg/kg</td>
<td>50% death in a few hours</td>
<td>Gull, Isenberg, and Bryan (1970)</td>
</tr>
<tr>
<td>Mice</td>
<td>Oral</td>
<td>1000 mg/kg</td>
<td>Nontoxic</td>
<td>Nishie, Gumbmann, and Keyl (1971)</td>
</tr>
<tr>
<td></td>
<td>Intraperitoneal</td>
<td>42 ± 1.8 mg/kg</td>
<td>50% death in 7 days</td>
<td>Nishie, Gumbmann, and Keyl (1971)</td>
</tr>
<tr>
<td></td>
<td>Intraperitoneal</td>
<td>10 mg/kg</td>
<td>Toxic(^c)</td>
<td>Patil et al. (1972)</td>
</tr>
<tr>
<td>Experiment</td>
<td>Administration</td>
<td>Amount</td>
<td>Effect</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------------------</td>
<td>----------------------------------</td>
<td>----------------</td>
<td>---------------------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td></td>
<td>Intraperitoneal</td>
<td>32.3 mg/kg</td>
<td>50% death</td>
<td>Patil et al. (1972)</td>
</tr>
<tr>
<td></td>
<td>Intraperitoneal</td>
<td>50 mg/kg</td>
<td>Lethal</td>
<td>Patil et al. (1972)</td>
</tr>
<tr>
<td>Chick embryo</td>
<td>Injection into yolk sac</td>
<td>18.8 ± 1 mg/kg</td>
<td>50% mortality in 18 days</td>
<td>Nishie, Gumbmann, and Keyl (1971)</td>
</tr>
<tr>
<td>(4 day-old)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>Intraperitoneal</td>
<td>20 mg/kg</td>
<td>Overnight death</td>
<td>Nishie, Gumbmann, and Keyl (1971)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Death in 2.5-24 hours, recovery if survived for at least 24 hours</td>
<td>Patil et al. (1972)</td>
</tr>
<tr>
<td></td>
<td>Intraperitoneal</td>
<td>30 mg/kg</td>
<td>Death in 6.25 hours</td>
<td>Nishie, Gumbmann, and Keyl (1971)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Death in 50 minutes</td>
<td>Patil et al. (1972)</td>
</tr>
<tr>
<td></td>
<td>Intravenous</td>
<td>10 mg/kg</td>
<td>Death in 2 minutes</td>
<td>Nishie, Gumbmann, and Keyl (1971)</td>
</tr>
</tbody>
</table>

*a In a case of potato poisoning (total glycoalkaloid).

*b Determined from potatoes consumed (total glycoalkaloid).

*c General symptoms of food poisoning.
Mycotoxicity of glycoalkaloids

In 1968 several investigators demonstrated the mycotoxic properties of glycoalkaloids. Low concentrations of glycoalkaloids (0.1-0.5 mg ml\(^{-1}\)) inhibited the growth of *Fusarium solani* hyphae by 70-90\% (Ozeretskovskaya et al., 1968). Allen and Kuc (1968) reported that at least 90\% of the mycotoxicity exhibited by potato peel extracts to *Helminthosporium carbonium* was due to solanine and chaconine. A natural mixture of glycoalkaloids (solanidine, solanine, and chaconine) at a concentration of 250 mg l\(^{-1}\) significantly inhibited the growth of *Alternaria solani* (Sinden et al., 1973). Solanine is also toxic to *Trichoderma viride* and this toxicity has been utilized in a bioassay technique. Patil et al. (1972) indicated that the LC\(_{50}\) for *T. viride* was 1.022 mg solanine ml\(^{-1}\).

Toxicity of blighted potatoes

In 1972 Renwick expounded a theory implying that two severe birth defects, spina bifida cystica and anencephaly, could be a consequence of a woman consuming blighted potatoes during her first month of pregnancy. The specific character of the substance present in blighted potatoes which induced the birth defects has not been elucidated. Further study was done by Poswillo et al. (1972) to establish a relationship between blighted potatoes and anencephaly. Although gross abnormalities in 4 of 11 fetuses in six animals and severe teratogenic defects in those conceived after a prolonged period of consumption of a blighted potato concentrate were found, no cases of anencephaly or spina bifida were reported. Epidemiological evidence indicates a
relationship exists between the consumption of blighted potatoes and the incidence of spina bifida and anencephaly (Remwick, 1972; Answell, 1972).
METHODS AND MATERIALS

Microorganisms and plant material

*Phytophthora infestans* (Mont.) de Bary race 1.2.4 was obtained from the culture collection of Dr. M. T. Wu, Department of Nutrition and Food Science, Utah State University. It was maintained on lima bean agar slants, incubated at 21°C, and stored at 4°C. Cultures were transferred every 2 weeks. The microorganisms used in the toxicity testing of glycoalkaloids were obtained from the culture collection of the Biology Department, Utah State University. These included *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter aerogenes*, *Erwinia amylovora*, *Serratia marcescens*, *Salmonella typhimurium*, *Streptococcus fecalis*, *Pseudomonas* sp. (viscosum), *P. fluorescens*, *P. fluorescens* Biotype IV (lemonnierii), *P. fluorescens* Biotype G, and *Xanthomonas campestris*.

Commercial seed potatoes of the cultivar Russett Burbank were purchased from a local grower. Tubers were washed and stored at 4°C until planting. Tubers were held in the dark at 20°C for 3 days before planting in order to break dormancy and enhance sprouting.

Media and chemicals

Czapek Dox agar, lima bean agar, plate count agar, potato dextrose agar, Sabouraud dextrose agar, and Sabouraud maltose agar were purchased from Difco Laboratories, Detroit, Michigan. Chick peas and rye seeds were bought from a local seed store. Chemicals utilized in the medium development and nutritional studies were all reagent.
grade and purchased from Sigma Chemical Company, St. Louis, Missouri. The organic chemicals used included: DL-alanine, L-alanine, arabino­se, L-arginine, L-asparagine, L-cystine, egg albumin, fructose, galactose, glucose, lactose, maltose, mannitol, peptone, L­
phenylalanine, potato extract, L-proline, skim milk, sodium acetate, sodium citrate, sodium oxalate, starch, sucrose, thiamin, and urea. The inorganic chemicals utilized in these studies included: ammonium chloride, calcium nitrate, cobalt (II) nitrate, copper (II) nitrate, iron (II) nitrate, magnesium sulfate, manganese (II) nitrate, nickel (II) nitrate, potassium dihydrogen phosphate, potassium nitrate, sodium chloride, sodium dihydrogen phosphate, sodium nitrate, and sodium sulfate.

Preparation of culture for inoculation

Stock cultures of P. infestans race 1.2.4 were prepared as inoculum by adding 10 ml of sterile 0.1% (w/v) peptone water to the agar slants and gently emulsifying the mycelium from the slant. The mass of inoculum was standardized by addition of material from the washed slant until the optical density of the suspension was 0.6 at 580 nm.

Development of an enriched basal medium

The commercially prepared media—potato dextrose agar, Sabouraud dextrose agar, Sabouraud maltose agar, and Czapek Dox agar—all enriched with thiamin, were employed in the initial screening of P. infestans for level of growth. French's (1953) synthetic medium, which consists of 25.0 g glucose, 1.0 g KNO₃, 0.5 g KH₂PO₄, 0.25 g MgSO₄·7H₂O, and 1.0 mg thiamin per liter of distilled water, was also
utilized in the initial screening. In order to determine an energy source for an enriched basal medium, various carbon sources were substituted for the glucose in French's medium including arabinose, fructose, galactose, lactose, maltose, mannitol, sodium acetate, sodium citrate, sodium oxalate, starch, and sucrose in the concentration range of 1.0-7.5% (w/v). When a positive growth response was demonstrated with increasing concentration of carbon source, additional study was conducted employing the carbon sources in the concentration range of 8.0-12.0% (w/v). The same carbon sources were utilized with ammonium chloride as the inorganic nitrogen source in place of potassium nitrate. Further study was carried on when *P. infestans* responded in a positive manner to increasing concentration of the energy source. Both the carbon and the nitrogen source in French's medium were replaced with several organic nitrogen sources in the concentration range of 1.0-7.5% (w/v). These compounds included L-alanine, L-arginine, L-asparagine, L-cystine, egg albumin, peptone, L-phenylalanine, potato extract, L-proline, skim milk, and urea. After statistical analysis of the data, 9.0% maltose was chosen as the carbon source with KNO₃ as the inorganic nitrogen source. Following the selection, development of the medium was further pursued by enrichment with one of the following amino acids: DL-alanine, L-proline, or a 1:1 (w:w) combination of the two. These enrichments were added to the basal medium in the concentration range of 0.5-3.0% (w/v). All media were in 50 ml volumes in 250 Erlenmeyer flasks. One-half ml of the standardized mycelium suspension was utilized to inoculate 50 ml of the test media. All media were sterilized at 121°C.
for 15 minutes, inoculated, and incubated in the dark in stationary culture at 21°C for 28 days.

*Phytophthora infestans* race 1.2.4 was also cultured on rye seed medium and chick pea medium. The rye seed medium (Hodgson, 1969) was prepared by steaming 100 g rye seeds in 500 ml distilled water for 30 minutes. This mixture was filtered through a double layer of cheesecloth, 5 g glucose were added to the extract, and the solution was diluted to 1 liter. The chick pea medium was prepared by soaking 100 g chick peas in distilled water overnight, draining them, and removing the testa by hand. One hundred ml or grams of media were placed in 500 ml Erlenmeyer flasks and sterilized at 121°C for 15 minutes. One ml of mycelium suspension was employed to inoculate 100 ml or grams of medium. These cultures were inoculated, and incubated in dark, stationary culture at 21°C for 21-35 days.

**Inorganic nutrition of *Phytophthora infestans***

The enriched basal medium developed in the above section was utilized in this portion of the study as a control medium. Sodium salts of the macronutrients were substituted for the potassium and magnesium salts of the macronutrients in the control medium. Potassium nitrate was replaced by sodium nitrate in the concentration range of 0.025-0.5% (w/v); potassium dihydrogen phosphate was substituted by sodium dihydrogen phosphate in the concentration range of 0.001-0.4% (w/v); and magnesium sulfate was replaced by sodium sulfate in the concentration range of 0.001-0.2% (w/v). These substitutions were carried out one nutrient at a time. Sodium chloride and sodium
acetate were individually added to the medium in the concentration range of 0.001-0.4% (w/v). To investigate the micronutrient requirements of *P. infestans* the nitrate salts of calcium, cobalt, copper, iron, manganese, and nickel were singly added to the medium in the concentration range of 0.0001 mg l\(^{-1}\) to 5.0 mg l\(^{-1}\). All chemicals utilized in the micronutrient study were analytical grade and the media were prepared with double glass distilled water. All test media were in 50 ml volumes in 250 ml Erlenmeyer flasks, sterilized at 121°C, inoculated with 0.5 ml of the mycelium suspension, incubated in dark, stationary culture at 21°C for 28 days.

**Harvesting of mycelium**

Mycelium was harvested by filtering the test media through pre-labeled, preweighed Whatman #2 filters. Traces of media were removed from the filters by washing with distilled water. The filters and mycelium were air-dried at 25°C to a constant weight. The dry weight of the mycelium per 50 ml test media was calculated. The filtrate from each sample was stored at 4°C for later glycoalkaloid analysis.

**Glycoalkaloid analysis**

Glycoalkaloids were extracted from the test media according to the procedures of Gull and Isenberg (1960) with some modifications. The alkaloids were extracted from the filtrate by blending in a Waring blender with 40 ml of hot ethanol for 4 minutes and repeating this process two more times using a total of 120 ml of alcohol. This mixture was filtered to remove any insoluble material and the filtrate was evaporated to near dryness in an evaporating dish. The residue was dissolved in 15 ml of 5% sulfuric acid and filtered through fluted
filter paper into a 50 ml glass centrifuge tube. The evaporating dish was washed with 10 ml of 5% sulfuric acid; this was filtered, and added to the glass centrifuge tube. The tube was immersed in an ice water bath and concentrated ammonium hydroxide was added until the pH of the solution exceeded 9.5. This basic solution was heated to 80°C in a hot water bath for one hour to facilitate the precipitation of glycoalkaloids. The resultant suspension was stored at 4°C overnight and the following day was centrifuged at 5,000 rpm for 30 minutes. The supernatant was decanted and the precipitate was washed with 1.0% ammonium hydroxide. This suspension was centrifuged, the supernatant decanted, and the precipitate dissolved in 1.0% sulfuric acid with dilution to 100 ml in a volumetric flask.

The amount of glycoalkaloids was quantified by development of measurable color in a 2.5 ml aliquot of the diluted solution. Color development was accomplished by adding 5 ml concentrated sulfuric acid dropwise over a period of 3 minutes with vigorous shaking followed by 2.5 ml of 1.0% formaldehyde added in the same manner over a period of 2 minutes. The above process was accomplished by utilizing an automatic shaking device equipped with special reservoir burettes (Wu and Salunkhe, 1976). The reaction mixture was incubated at room temperature (25°C) for 105 minutes to allow for complete color development which was measured at 565 nm on a Bausch and Lomb Spectronic-20 spectrophotometer. A standard curve was prepared employing standard concentrations of solanine (Figure 4) and the concentration of glycoalkaloids was calculated from the simple regression equation \( \hat{Y} = 0.425 + 49.132X \) where \( X \) = optical density and \( \hat{Y} = \) mg total glycoalkaloids per 100 ml.
Figure 4. Solanine standard curve.
\[
\hat{Y} = 0.425 + 49.132 \bar{X}
\]
Test media which were positive for glycoalkaloids were further analyzed by thin-layer chromatography (TLC) according to the procedures of Zitnak (1968). The samples were evaporated to near dryness and the residue was dissolved in 10 ml absolute methanol. Aliquots were applied to silica gel plates and developed by ascending chromatography utilizing a solvent system of butanol, acetic acid, and water (10:3:1). After drying the plates were sprayed with antimony trichloride in acetic acid (2:1) and heated for 10 minutes at 100°-110°C. With this treatment glycoalkaloids produced a color reaction. Standard solutions of solanine, chaconine, and solanidine were utilized as references.

Resistance of potato foliage to *Phytophthora infestans*

Sprouted seed potatoes of cultivar Russett Burbank were hand planted in loam soil in standard size greenhouse pots. The potatoes were grown indoors at 23°-27°C and were situated 1.5 m from the light source. Light was obtained from one bank of fluorescent tubes 2.44 m long (ITT/F96T 121 CW cool white). The light intensity at the surface of the pots due to this arrangement was 1076 lux. The plants were watered as necessary. Plants were fertilized with nitrogen, in the form of potassium nitrate or phosphate in the form of potassium dihydrogen phosphate. Fertilizer solutions were prepared in the concentration range of 1.0-25.0% (w/v). One hundred ml of fertilizer solution was applied once per potato plant. After the potatoes reached maturity leaflets were sampled for testing of their resistance to infection by *P. infestans* race 1.2.4. Leaflets selected for testing were fully developed and had good turgidity. Terminal
leaflet samples were avoided and three samples were removed from each plant representing three leaf positions: top, intermediate, and bottom. Individual detached leaflets were placed in 12.5 cm sterile glass Petri plates containing moistened filter paper. Each leaflet was inoculated with *P. infestans* by pipetting two separate drops of the standardized mycelium suspension to the top surface of the leaf. The inoculated leaflets were incubated in the dark at 21°C for 5 days. Sterile distilled water was added to the filter paper as needed to maintain the relative humidity within the Petri plate. Susceptibility of the foliage to *P. infestans* was determined by the size of the lesion. The diameter of each lesion was measured in mm along the axis parallel to the main vein of the leaflet on the fifth day of incubation.

**Toxicity of steroid glycoalkaloids to bacteria**

The bacteria used in this study were grown on plate count agar slants. Cultures of bacteria were prepared for use as an inoculum by adding 0.1% peptone water to the slants and gently washing the cells from the surface of the agar. Serial dilutions of bacterial suspensions were carried out and pour plates were made. The dilution containing $10^5$-$10^6$ viable bacteria per ml was utilized as the standardized inoculum in this investigation. One-tenth ml of the inoculum was spread on the surface of the plate count agar. These plates were divided into quarters and 1.25 cm absorbent paper assay discs were centered in each quadrant. Glycoalkaloids were dissolved in sterile distilled water at pH 5.8 in the concentration range of 1.0-200.0 mg total glycoalkaloids per 100 ml. Sterile distilled water at pH
5.8 was employed as a control. One-tenth ml of a glycoalkaloid concentration was pipetted per assay disc. Plates containing glycoalkaloids were prepared in duplicate. Plates were incubated at 30°C for 72 hours and were examined for inhibition of growth every 12 hours.

Statistical analysis

All experiments were arranged in a completely randomized design. The investigations concerning development of the enriched basal medium were carried out in four replicates. Three replicates with 10 flasks in each were performed during the nutritional studies. Experiments on foliage susceptibility of *P. infestans* were done in five replicates with two determinations per replicate. Toxicity studies were carried out in duplicate. Statistical analysis was computed and the means were compared according to the least significance difference (LSD) procedure (Steel and Lorrie, 1966) wherever possible.
RESULTS

Development of an enriched basal medium

Growth of *Phytophthora infestans*. During initial screening of media for growth of *P. infestans*, no growth was detected in potato dextrose agar, Sabouraud dextrose agar, Sabouraud maltose agar, and Czapek Dox agar, all enriched with thiamin. Minimal growth, 0.018 g dry weight mycelium per 50 ml medium, occurred in unmodified French's medium. When fructose, arabinose, mannitol, starch, sodium acetate, sodium citrate, and sodium oxalate were substituted for the glucose in French's medium, with KNO₃ as the inorganic nitrogen source, no measurable growth was observed. With maltose, sucrose, galactose, and lactose *P. infestans* race 1.2.4 yielded varying amounts of growth. Figure 5 illustrates the growth response of *P. infestans* in media containing the above nutrients in the concentration range of 1.0-7.5% (w/v). The analysis of variance, presented in Appendix Table 4, indicates that with the exception of the mycelium yields obtained with 1.0% maltose, 5.0% galactose, and 7.5% galactose, all other mycelium yields were significantly higher than the minimal growth observed in French's unmodified medium. The highest level of growth of *P. infestans*, 0.325 g dry weight mycelium per 50 ml medium, was obtained when 5.0% lactose was utilized as the carbon source. Upon increasing the concentrations of these carbon sources to 8.0-12.0% (w/v), elevated levels of growth were found with maltose, sucrose, and
Figure 5. Growth of *Phytophthora infestans* race 1.2.4 after 28 days at 21°C in French's medium with various carbon sources in the concentration range of 1.0-7.5% (w/v) with KNO₃ as the inorganic nitrogen source with 95% confidence intervals. (See Appendix Table 4 for statistical data.)
lactose. These data are plotted in Figure 6 and statistical analysis of these data is presented in Appendix Table 5.

When ammonium chloride was utilized as the inorganic source, *P. infestans* race 1.2.4 failed to grow on starch, sodium acetate, sodium citrate, and sodium oxalate as carbon sources, but produced varying amounts of growth on glucose, maltose, fructose, galactose, sucrose, lactose, arabinose, and mannitol. These data are plotted in Figure 7. Mycelium yields with 7.5% galactose, 7.5% glucose, and 7.5% maltose were significantly higher (Appendix Table 6) than the quantity of growth detected in French's unmodified medium and in all other media containing NH₄Cl as the inorganic nitrogen source. When the concentrations of these carbohydrates were increased to 8.0-12.0% (w/v), elevated levels of growth were observed with glucose, maltose, lactose, galactose, and sucrose. These data are presented in Figure 8. Growth levels of *P. infestans* with 12.0% glucose, 11.0% lactose, and 12.0% lactose were significantly higher than the mycelium mass obtained with all other concentrations of carbohydrates (Appendix Table 7). In general, the amount of growth was greater when potassium nitrate was utilized as the inorganic nitrogen source than when ammonium chloride was employed. The highest level of growth obtained with KNO₃ was 0.485 g mycelium dry weight per 50 ml medium with 11.0% maltose, whereas the highest level of growth obtained with NH₄Cl was 0.393 g mycelium dry weight per 50 ml medium with 12.0% glucose.

Upon substitution of organic nitrogen compounds for the carbon and inorganic nitrogen in French's medium, varying amounts of growth of the fungus were observed in peptone, skim milk, L-arginine, L-proline, and L-alanine. These data are presented in Table 2.
Figure 6. Growth of *Phytophthora infestans* race 1.2.4 after 28 days at 21°C in French's medium with various carbon sources in the concentration range of 8.0-12.0 (w/v) with KNO₃ as the inorganic nitrogen source with 95% confidence intervals. (See Appendix Table 5 for statistical data.)
G MYCELIUM DRY WEIGHT/50ml

0.6

0.5

0.4

0.3

0.2

0.1

0.0

CARBOHYDRATE CONCENTRATION (%)

8.0

9.0

10.0

11.0

12.0

MALTOSE
LACTOSE
SUCROSE
Figure 7. Growth of *Phytophthora infestans* race 1.2.4 after 28 days at 21°C in French's medium with various carbon sources in the concentration range of 1.0-7.5% (w/v) with NH₄Cl as the inorganic nitrogen source with 95% confidence intervals. (See Appendix Table 6 for statistical data.)
Figure 8. Growth of *Phytophthora infestans* race 1.2.4 after 28 days at 21°C in French's medium with various carbon sources in the concentration range of 8.0-12.0% (w/v) with NH₄Cl as the inorganic nitrogen source with 95% confidence intervals. (See Appendix Table 7 for statistical data.)
Table 2. Growth of *Phytophthora infestans* race 1.2.4 after 28 days at 21°C in French's medium with various organic carbon and nitrogen sources in the concentration range of 1.0-7.5% (w/v)

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Peptone</th>
<th>Skim milk</th>
<th>L-alanine</th>
<th>L-arginine</th>
<th>L-proline</th>
<th>Control(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.037(^b)</td>
<td>0.070</td>
<td>0.048</td>
<td>0.0</td>
<td>0.045</td>
<td>0.018</td>
</tr>
<tr>
<td>2.5</td>
<td>0.210</td>
<td>0.122</td>
<td>0.083</td>
<td>0.0</td>
<td>0.072</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>0.384</td>
<td>0.398</td>
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<tr>
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<td>0.0</td>
<td>0.221</td>
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</table>

\(^a\)French's unmodified medium.
\(^b\)Grams dry weight mycelium per 50 ml medium.
measurable growth was detected in potato extract, L-phenylalanine, L-asparagine, L-cystine, urea, and egg albumin.

After examination of the data, maltose at the 9.0% level was chosen as the carbon source since it was the lowest level of carbohydrate which demonstrated a significant level of growth. Potassium nitrate was chosen as the inorganic nitrogen source because growth yields were higher when KNO₃ was utilized than when NH₄Cl was employed.

Further enrichment of the basal medium was carried out by supplementing the medium with organic nitrogen compounds. Although the highest levels of growth were observed with medium containing skim milk or peptone, these compounds were not utilized as supplements in the basal medium because of their chemical variability. Alanine and proline were selected as enrichments since *P. infestans* grew in these compounds at the concentration level of 1.0 and 2.5% (w/v). These substances were added individually and in a mixture of the two in the concentration range of 0.5-3.0% (w/v). The highest level of growth was achieved when a concentration of 2.0% DL-alanine and 2.0% L-proline was employed. These data are presented in Table 3.

The enriched basal medium utilized in further experiments in this investigation consisted of: 90.0 g maltose, 20.0 g DL-alanine, 20.0 g L-proline, 1.0 g KNO₃, 0.5 g KH₂PO₄, 0.25 g MgSO₄·7H₂O, and 1.0 mg thiamin per liter of distilled water. *P. infestans* was grown in this medium in three replicates with 10 flasks in each replicate. The average level of growth was 0.586 g dry weight mycelium per 50 ml medium.
Table 3. Growth of *Phytophthora infestans* race 1.2.4 after 28 days at 21°C in basal medium with enrichments in the concentration range of 0.5-3.0% (w/v)

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>DL-alanine</th>
<th>L-proline</th>
<th>1:1 Combination^a^</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.600^b^</td>
<td>0.649</td>
<td>0.891</td>
</tr>
<tr>
<td>1.0</td>
<td>0.681</td>
<td>0.841</td>
<td>0.773</td>
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<td>1.5</td>
<td>0.583</td>
<td>0.871</td>
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<td>2.0</td>
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<td>0.887</td>
<td>1.067</td>
</tr>
<tr>
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<td>0.785</td>
<td>1.025</td>
</tr>
<tr>
<td>3.0</td>
<td>0.551</td>
<td>0.734</td>
<td>0.955</td>
</tr>
</tbody>
</table>

^a^1:1 Combination at a concentration of 0.5% contains 0.5% DL-alanine and 0.5% L-proline.

^b^Grams dry weight mycelium per 50 ml medium.

**Production of glycoalkaloids by *Phytophthora infestans***

Enriched basal medium. When samples from filtrates of the above medium in which *P. infestans* was cultured were analyzed for steroid glycoalkaloids, the results indicate that 62.5% of the samples were positive for glycoalkaloids. The average level of alkaloids in the three replicates was 0.852 mg total glycoalkaloids per 25 ml medium with a range of 0.0 to approximately 5.9 mg total glycoalkaloids per 25 ml medium. The results indicated that a minimal level of growth of 0.35-0.4 g dry weight mycelium/50 ml medium was necessary before a measurable concentration of glycoalkaloids could be demonstrated.

Synthesis of glycoalkaloids by *P. infestans* race 1.2.4 is shown in Figure 9. Thin-layer chromatography of the 15 positive samples revealed that the major glycoalkaloid constituents were solanidine, which was present in all the samples, and solanine, which was present in three of the samples.
Figure 9. Growth of *Phytophthora infestans* race 1.2.4 and the synthesis of steroid glycoalkaloids in enriched basal medium after 28 days at 21°C.
Semisynthetic medium. In 10 samples of rye seed medium, abundant growth of *P. infestans* race 1.2.4 developed after 14 days incubation at 21°C, but no glycoalkaloids were detected in the medium. In chick pea medium, mycelium completely covered all exposed surfaces of the chick peas after 28 days at 21°C. In 25 g aliquots of the chick pea medium incubated for 28 days at 21°C, no glycoalkaloids were detected, whereas in 25 g samples of chick pea medium incubated for 35 days at 21°C, 50% of the samples were positive for glycoalkaloids. The average concentration of alkaloids in chick pea medium was 0.67 mg per 25 g chick peas. Ten samples of lima bean agar on which *P. infestans* race 1.2.4 was cultured for 28 days at 21°C were screened for glycoalkaloids. None of the samples were positive.

**Effects of nutrients on the growth of *Phytophthora infestans***

**Macronutrients.** In the medium containing sodium salts of the macronutrients, none of the mycelium yields were greater than the yield in the control (basal medium). These data are presented in Figure 10. However, the highest levels of growth detected in the media containing sodium salts were not significantly lower at the 95% statistical level (Appendix Table 8) than the level detected in the control medium. The highest levels of growth were observed when the following macronutrient concentrations were utilized: 0.25-0.1%, 0.4-0.5% NaNO₃, 0.02-0.04% NaH₂PO₄, 0.02-0.05%, 0.2% NaCl, and 0.01-0.04% Na₂SO₄. Sodium acetate appears to be inhibitory to *P. infestans* since all mycelium yields were significantly lower than the amount of mycelium in the control medium.
Figure 10. Growth of *Phytophthora infestans* race 1.2.4 after 28 days at 21°C in enriched basal medium with individually substituted sodium salts in the concentration range of 0.001-0.5% (w/v) with 95% confidence intervals. (Statistical data are presented in Appendix Table 8.)
Micronutrients. Addition of microelements to the basal medium showed varied results. These data are illustrated in Figure 11. The amount of mycelium detected in the control medium in the micronutrient study (Figure 11) was lower than the level of mycelium observed in the control medium in the macronutrient study (Figure 10). This may be attributable to a decrease in the level of contaminating microelements in the basal medium of the micronutrient study due to use of double glass distilled water and analytical grade chemicals in preparation of the micronutrient control medium. Growth of the fungus was stimulated by addition of iron in the ferrous state with significantly higher levels of *P. infestans* obtained when iron was added to the medium in the concentration range of 1.0-5.0 mg L⁻¹ (Appendix Table 9). Growth of *P. infestans* was significantly inhibited by all concentrations of copper, cobalt, nickel, manganese, and by concentrations of calcium that exceeded 0.001 mg L⁻¹.

Effects of nutrients on synthesis of glycoalkaloids by *Phytophthora infestans*

Macronutrients. Synthesis of glycoalkaloids by *P. infestans* race 1.2.4 in media containing sodium salts of the macronutrients is illustrated in Figures 12 and 13. Figure 12 refers to the percentage of samples that were positive for glycoalkaloids in media containing sodium salts of the macronutrients and Figure 13 illustrates the average concentration of alkaloids detected in the test media. The average level of glycoalkaloids detected in the control medium was 0.85 mg total glycoalkaloids per 25 ml medium with 62.5% of the samples positive for alkaloids. Although the percentage of samples positive
Figure 11. Growth of *Phytophthora infestans* race 1.2.4 after 28 days at 21°C in enriched basal medium with the nitrate salts of micronutrients in the concentration range of 0.0001-5.0 mg l⁻¹ with 95% confidence intervals. (Statistical data are presented in Appendix Table 9.)
Figure 12. Percentage of samples containing steroid glycoalkaloids synthesized by *Phytophthora infestans* race 1.2.4 after 28 days at 21°C in enriched basal medium with substituted sodium salts of macronutrients in the concentration range of 0.001-0.5% (w/v) with 95% confidence intervals. (Statistical data are presented in Appendix Table 10.)
MACRONUTRIENT CONCENTRATION (%)

SAMPES CONTAINING ALKALOIDS (%)
Figure 13. Quantity of steroid glycoalkaloids synthesized by *Phytophthora infestans* race 1.2.4 after 28 days at 21°C in enriched basal medium with substituted sodium salts of the macronutrients in the concentration range of 0.001-0.5% (w/v) with 95% confidence intervals. (Statistical data are presented in Appendix Table 11.)
MG TOTAL GLYCOALKALOIDS/25 ML MEDIUM

MACRONUTRIENT CONCENTRATION (%)
for glycoalkaloids in the control was higher than the percentage of positive samples in media containing sodium salts of the macronutrients, statistical analysis of the data indicates that there is no significant difference between these results (Appendix Table 10). The average level of glycoalkaloids decreased significantly (Appendix Table 11) upon substitution of sodium salts for the individual potassium and magnesium salts or addition of sodium chloride. Although variations in the quantity of glycoalkaloids produced in media containing sodium salts were observed, these differences were not significant at the 95% statistical level.

In order to determine the effect of each sodium salt on the synthesis of glycoalkaloids by *P. infestans*, simple regression analysis was performed on (1) the effect of nutrient concentration and (2) the effect of the level growth of *P. infestans* on the amount of glycoalkaloids produced by the fungus. Results indicate that the concentration of NaN03 or NaCl in the media has little effect on the amount of alkaloids synthesized by the fungus. In media containing NaH2PO4 or Na2SO4, as their concentrations increased the level of glycoalkaloids synthesized by *P. infestans* increased in accordance with the equations, \( \hat{y} = 0.1616 + 0.0396\bar{x} \) and \( \hat{y} = 0.73 + 0.0362\bar{x} \), respectively, where \( \hat{y} = \text{mg total glycoalkaloids per 25 ml medium} \) and \( \bar{x} = \text{concentration of macronutrient in percent} \). The same trend was demonstrated when data concerning the effect of nutrient concentration on the percentage of positive samples was analyzed. Analysis of results relating the level of glycoalkaloids produced by the fungus with the mycelium yield in media containing various concentrations of sodium salts showed that in various levels of NaCl the yield of
mycelium had little influence on the quantity of glycoalkaloids synthesized by the fungus. But when a range of NaH$_2$PO$_4$ or Na$_2$SO$_4$ was utilized in the media the quantity of glycoalkaloids produced by the fungus decreased as the level of growth of *P. infestans* increased in relation to the linear equations, $\hat{y} = 0.1278 - 0.224X$ and $\hat{y} = 0.1919 - 0.3224X$, respectively, where $\hat{y} =$ mg total glycoalkaloids per 25 ml medium and $X =$ g dry weight mycelium per 50 ml medium. When *P. infestans* was grown in media containing differing concentrations of NaN$_3$, the glycoalkaloid concentration increased as the level of growth of *P. infestans* increased according to the equation $\hat{y} = 0.1582 + 0.602X$, where $\hat{y} =$ mg total glycoalkaloids per 25 ml medium and $X =$ g dry weight mycelium per 50 ml medium. The same pattern was demonstrated when growth and concentration parameters were analyzed with the percentage of samples positive for steroid glycoalkaloids.

Analysis of all positive samples from the control medium and the test media containing sodium salts of the macronutrients was carried out by thin-layer chromatography. Results of this analysis showed that the basal medium contained both solanidine, present in 100% of the samples, and solanine, present in 20% of the samples. In media containing sodium salts, only solanidine was present.

**Micronutrients.** No glycoalkaloids were detected in media containing copper, cobalt, calcium, nickel, or manganese. Upon addition of iron to the basal medium, glycoalkaloids were found to be produced by *P. infestans* at all levels of iron except 5.0 mg l$^{-1}$. The highest percentage of positive samples (Figure 14) and the highest concentration of glycoalkaloids (Figure 15) were detected when 0.001 mg l$^{-1}$ iron was added to the basal medium. Statistical analysis of the data
Figure 14. Percentage of samples containing steroid glycoalkaloids synthesized by *Phytophthora infestans* race 1.2.4 after 28 days at 21°C in enriched basal medium with ferrous iron in the concentration range of 0.001-5.0 mg l⁻¹ with 95% confidence intervals. (Statistical data are presented in Appendix Table 12.)
Figure 15. Quantity of steroid glycoalkaloids synthesized by *Phytophthora infestans* race 1.2.4 after 28 days at 21°C in enriched basal medium with ferrous iron in the concentration range of 0.001-5.0 mg l⁻¹ with 95% confidence intervals. (Statistical data are presented in Appendix Table 13.)
from Figure 14 indicates that the percentage of samples positive for glycoalkaloids in the control medium and in medium containing 0.001 mg l\(^{-1}\) iron were significantly higher than the percentage of positive samples observed in other iron containing media (Appendix Table 12). Statistical examination of the data from Figure 15 indicates that the level of glycoalkaloids in the control medium was significantly higher than the concentration observed in the media containing Ferrous iron (Appendix Table 13), although in media containing 0.001 or 0.01 mg l\(^{-1}\) iron the quantity of alkaloids synthesized by \textit{P. infestans} was significantly higher than the amount produced in media containing higher levels of iron.

Regression analysis illustrates that as the concentration of iron increases the quantity of glycoalkaloids synthesized by the fungus declines in accordance with the equation \(\hat{y} = 3.341 - 7.819\bar{x}\), where \(\hat{y}\) = mg total glycoalkaloids per 25 ml medium and \(\bar{x}\) = concentration of iron in mg per liter. As varying concentrations of iron were added to the basal medium, the level of glycoalkaloids produced by the fungus decreased as the mycelium yield increased. The relationship of these parameters is shown by the simple regression equation \(\hat{y} = 0.4462 - 0.428\bar{x}\), where \(\hat{y}\) = mg total glycoalkaloids per 25 ml medium and \(\bar{x}\) = g dry weight mycelium per 50 ml medium. The same trend is demonstrated with data concerning the percentage of positive samples.

\textbf{Effect of fertilizer on the susceptibility of potato foliage to \textit{Phytophthora infestans}}

\textbf{Nitrate and phosphate fertilizers.} As different levels of nitrate or phosphate fertilizer were applied to Russet Burbank potato
plants the susceptibility of foliage to infection by \textit{P. infestans} race 1.2.4 was influenced. Results of this study are illustrated in Figure 16. Application of 20.0\% or 25.0\% potassium nitrate fertilizer to potato plants significantly increased the susceptibility of potato leaves to infection by \textit{P. infestans} race 1.2.4 (Appendix Table 14). The level of potassium dihydrogen phosphate applied to potato plants had no significant effect on the resistance of potato foliage to infection by \textit{P. infestans}.

\textbf{Glycoalkaloid toxicity}

\textbf{Bacteria.} When common enteric and saprophytic bacteria were grown in the presence of steroid glycoalkaloids in the concentration range of 1.0-200.0 mg total glycoalkaloids per 100 ml, no inhibitory effect was demonstrated on the growth of these microorganisms. The bacteria utilized in this study included: \textit{Streptococcus fecalis}, \textit{Staphylococcus aureus}, \textit{Escherichia coli}, \textit{Enterobacter aerogenes}, \textit{Erwinia amylovora}, \textit{Serratia marcescens}, \textit{Salmonella typhimurium}, \textit{Pseudomonas fluorescens}, \textit{P. fluorescens Biotype IV (lemonnieri)}, \textit{P. fluorescens Biotype G (geniculata)}, \textit{Pseudomonas sp. (viscosum)}, and \textit{Xanthomonas campestris}. 
Figure 16. Effects of nitrate and phosphate fertilizers on the susceptibility of Russet Burbank potato foliage to infection with *Phytophthora infestans* race 1.2.4 with 95% confidence intervals. (Statistical data are presented in Appendix Table 14.)
DISCUSSION

Development of an enriched basal medium

Growth. *Phytophthora infestans* race 1.2.4 is a fastidious organism which seems to assimilate nitrogen in the nitrate form more readily than in the ammonium state as illustrated by the steeper growth response curves and overall higher mycelium yield produced when nitrate was employed as the inorganic nitrogen source.

In general higher levels of growth were observed when a disaccharide (sucrose, maltose, lactose) containing at least one glucose molecule was utilized as the carbon source in the medium. Glucose alone was not studied with nitrate, but glucose provided the greatest growth support when ammonium was the inorganic nitrogen source. The highest level of growth occurred with maltose as the carbon source.

Upon addition of the amino acids, DL-alanine and L-proline, to the basal medium, the average level of growth increased by approximately four times, suggesting that these amino acids can be synthesized by the fungus during growth but addition of these compounds greatly stimulates growth.

Some of the growth variability demonstrated by samples numbered 2, 3, 4, and 5 in Figure 5 may be due to inhibition by the glycoalkaloids produced since the effective dose of glycoalkaloids for 50% inhibition of growth of *Phytophthora infestans* (ED\(_{50}\)) is 6.25 mg glycoalkaloids per 25 ml medium (Shih et al., 1973). The wide range of glycoalkaloids values indicates involvement of unknown factors.
Glycoalkaloid production. The critical nature of the medium composition to support glycoalkaloid production is evident, since there was production in the enriched basal medium and in the chick pea medium, but not in the rye seed medium or in the lima bean medium. Although a growth level of 0.35-0.40 g dry weight mycelium was necessary before glycoalkaloids were detected in the medium, less growth may yield glycoalkaloids at concentrations below the detection limit.

Since glycoalkaloids are mycotoxic (Allen and Kuc, 1968; Ozeretskovskaya et al., 1968; Sinden et al., 1973), synthesis of these compounds may be a protective response of the potato to fungal infection. Locci and Kuc (1967) reported increased synthesis of glycoalkaloids by potatoes infected with Helminthosporium carboxonium and P. infestans. Ishizaka and Tomiyama (1972) observed large accumulations of glycoalkaloids in cut potatoes infected with P. infestans, but only small concentrations of glycoalkaloids in intact potato tissue. Increased glycoalkaloid synthesis which occurred in mechanically injured potatoes (Sinden, 1972) was probably due to weakened potato integument allowing for easier penetration of the potato by microorganisms.

Glycoalkaloids are detected in "healthy" potatoes in trace amounts, but it is possible that the presence of these compounds may be the result of a low, undetectable level of infection of P. infestans somewhere on the plant. Growing potatoes in axenic culture and analyzing the mature tubers for glycoalkaloids might indicate that uninfected potatoes do not synthesize glycoalkaloids. It is interesting to speculate on the possibility that coevolution, the joint evolution of two or more taxa that have a close ecological relationship but do
not exchange genes and in which reciprocal selective forces operate
to make the evolution of either taxon partially dependent upon the
evolution of the other (Ehrlich and Raven, 1964), may be the deter-
mining factor. Coevolution encompasses most forms of population
interaction, including parasitism. The ability of *P. infestans*
and/or the potato to synthesize glycoalkaloids may be due to the
long interrelationship of the two organisms during which coevolution
took place.

**Effects of inorganic nutrients on**
the growth of *Phytophthora infestans*

Since *P. infestans* has been a parasite of the Irish potato for
a considerable time, one would expect its nutritional requirements
to be related to the chemical composition of the potato. The raw
Irish potato contains per 100 g wet weight: 17.6 g carbohydrate,
2.1 g protein, 0.1 g fat, 407 mg potassium, 53 mg phosphate, 7 mg
calcium, 3 mg sodium, 0.6 mg iron, with a total ash content of 900 mg
(Watt and Merrill, 1963).

**Macronutrients.** The results indicate that *P. infestans* responds
well to phosphate and chloride at the 0.04% level with a decrease in
mycelium yield when these nutrients were increased to the 0.05%
level. This suggests that the 0.05% level of phosphate utilized in
the enriched basal medium may be somewhat inhibitory to the fungus.
The maximum amount of mycelium obtained when Na$_2$SO$_4$ was employed in
the medium was considerably lower than the maxima obtained with other
macronutrients. This may be due to the lack of magnesium in the
medium rather than an influence of sulfate, since magnesium is
important in cell metabolism as a cofactor for many enzymes and in ribosomal function.

**Micronutrients.** Stimulation of growth of *P. infestans* by the higher levels of iron tested in this study (2-5 mg l⁻¹) may be attributable to enhancement of the activity of heme proteins and other oxidative enzymes which utilize iron as a cofactor. Apparently the requirements of *P. infestans* for calcium, copper, cobalt, nickel, and manganese are lower than 0.0001 mg l⁻¹, since at this level and above some degree of inhibition of growth of *P. infestans* was observed.

**Effects of inorganic nutrients on the synthesis of glycoalkaloids by** *Phytophthora infestans*

Glycoalkaloids, members of a group of compounds known as secondary metabolites that occur in plants and microorganisms, are not known to serve any direct physiological function (Pianka, 1974). Most of these chemicals are not waste products, but are rather secondary substances produced by active synthesis. These compounds often contain nitrogen or other elements which are available to organisms in a limited supply, and it requires energy to synthesize secondary chemicals. Synthesis of glycoalkaloids by the potato is induced when the plant has been subjected to physiological stress, such as infection by *P. infestans*.

**Macronutrients.** The results indicated that synthesis of glyco-alkaloids by *P. infestans* race 1.2.4 was dependent upon the chemical composition of the medium, as illustrated by the significant decrease in the quantity of glycoalkaloids synthesized by the fungus when sodium salts were utilized in the medium. The enzyme systems involved
in the synthesis of glycoalkaloids are probably adapted to the chemical composition of the potato which contains a potassium to sodium ratio of approximately 400:1. In microorganisms, potassium has been shown to function in the cell as a compatible solute for enzyme stability (Brown, 1976); therefore, disruption of the potassium:sodium ratio of the cell may result in decreased activity of one or more of the enzymes involved in the synthesis of glycoalkaloids. With increasing concentrations of phosphate or sulfate in the medium, the quantity of glycoalkaloids produced by the fungus increased; this could be attributable to a higher degree of stress applied to the fungus by higher concentrations of these nutrients. The fungus may respond to a more stressful environment in the same manner as the potato; i.e., by production of higher levels of stress metabolites, such as steroid glycoalkaloids. Phosphate appears to have the most influence on the relationship between the amount of mycelium produced and the level of glycoalkaloids synthesized by the fungus. With the spectrum of phosphate concentrations utilized in this study, as the amount of mycelium increased the quantity of glycoalkaloids produced by the fungus declined. This resultant relationship may be due to an adjustment in the metabolism of the fungi from synthesis of glycoalkaloids to synthesis of factors which relate directly to growth. This shift in metabolic pathways may be due to an easing of stressful conditions when higher masses of mycelium are present.

Micronutrients. The results show that the highest level of glycoalkaloid production in media containing iron occurred with a low level of iron (0.001 mg l\(^{-1}\)) and is followed by a decline in the
quantity of glycoalkaloids synthesized by *P. infestans* as the concentration of iron in the medium increases. This phenomenon has been demonstrated in the synthesis of other toxic substances by microorganisms. Pappenheimer et al. (1962) observed that when the iron concentration reached 100 µg l\(^{-1}\) synthesis of toxin by *Corynebacterium diphtheria* reached its maximum and declined as the level of iron was increased to 500 µg l\(^{-1}\), where it became negligible.

**Effects of fertilizer on the susceptibility of potato foliage to *Phytophthora infestans***

**Nitrogen.** Most studies on the relationship of nutrition and the incidence of foliage blight have been conducted using field experiments (Langbein and Pehl, 1962; Linter, 1968) or growth of potatoes in nutrient solutions (Weindlmayer, 1965). The results of these investigations indicated that application of high levels of nitrogen increased the susceptibility of potato foliage to infection by *P. infestans*. In this investigation, which was carried out under more controlled conditions using potted plants, the results supported those obtained in prior field studies. This increased susceptibility has been attributed to an increase in leaf formation (Langbein and Pehl, 1962).

**Toxicity of glycoalkaloids**

**Bacteria.** Glycoalkaloids have been shown to be toxic to fungal pathogens (Conner, 1937; Allison, 1952; McKee, 1959), but have been shown to be ineffective against bacterial pathogens, such as *Pseudomonas* spp. and *Erwinia* spp. (McKee, 1959). The viability of these
microorganisms was not affected after several hours of exposure to 2000 mg solanine per liter. Paquin and LaChance (1963) showed that glycoalkaloids extracted from potatoes were bacteriostatic to Corynebacterium sepedonicum, causative agent of potato ring rot, although potato sprouts containing high levels of glycoalkaloids were extensively invaded by C. sepedonicum (Paquin, 1966). The results obtained in this study which indicated no inhibition of enteric or saprophytic bacteria by glycoalkaloids support the findings of other investigators.
CONCLUSION

In order to study the growth of *Phytophthora infestans* and production of steroid glycoalkaloids by the fungus under controlled conditions, a chemically defined medium was developed. This synthetic medium consisted of: 90.0 g maltose, 20.0 g DL-alanine, 20.0 g L-proline, 1.0 g KNO₃, 0.5 g KH₂PO₄, 0.25 g MgSO₄·7H₂O, and 1.0 mg thiamin per liter of distilled water. The average growth level of *P. infestans* in this defined medium was 0.586 g dry weight mycelium per 50 ml medium. Glycoalkaloids were synthesized by the fungus in 62.5% of the medium samples with an overall average concentration of 0.85 mg total glycoalkaloids per 25 ml medium. This enriched basal medium can be utilized by other researchers to further study the physiology of *P. infestans*.

When sodium salts of the macronutrients were substituted for the individual potassium and magnesium salts in the enriched basal medium, none of the mycelium yields were higher than the yield in the enriched basal medium and production of glycoalkaloids was significantly decreased. Of the macronutrients tested, phosphate appears to exert the most influence on the synthesis of glycoalkaloids by *P. infestans*. As the concentration of phosphate was raised the quantity of glycoalkaloids produced by the fungus increased.

These data indicate that the presence of sodium ions in the medium inhibits both growth of *P. infestans* and synthesis of glycoalkaloids by the fungus. These observations may be applied in
nutritional control of late potato blight by utilizing sodium salts of nitrate and/or phosphate, rather than potassium salts, as fertilizers.

Upon addition of micronutrients to the enriched basal medium, mycelium growth of *P. infestans* was stimulated by ferrous iron in the concentration range of 1.0-5.0 mg l⁻¹. Significant inhibition of fungal growth was observed with all concentrations of cobalt, copper, manganese, nickel, and by concentrations of calcium greater than 0.001 mg l⁻¹. These results indicate that media employed for culturing of *P. infestans* should contain ferrous iron in the level of 1.0-5.0 mg l⁻¹ and no other micronutrients. These observations indicate that addition of trace quantities of the micronutrients (cobalt, copper, nickel, and manganese) to macronutrient fertilizers may decrease the incidence of infection of potatoes by *P. infestans* due to an increased trace element level in potato tissues.

Synthesis of glycoalkaloids by *P. infestans* was inhibited by higher concentrations of iron (0.1-5.0 mg l⁻¹) with the highest concentration being detected at 0.001 mg l⁻¹. No glycoalkaloids were detected in media containing the other micronutrients tested.

Data from macronutrient and micronutrient studies illustrated that production of glycoalkaloids by *P. infestans* is critically dependent upon the chemical composition of the media. Therefore media utilized for production of glycoalkaloids by *P. infestans* should not contain any sodium salts or micronutrients.

Investigations concerning control of late potato blight through application of macronutrient fertilizers showed that phosphate and
low levels of nitrate had little effect on the resistance of potato foliage to infection by *P. infestans*. Higher levels of nitrate (20-25%) significantly decreased the resistance of potato leaves to infection by the late potato blight fungus. This may be due to an increase in leaf surface area, stimulated by high nitrogen levels, which facilitates infection of potato leaves by *P. infestans*. In *vitro* studies showed that cobalt, copper, manganese, and nickel inhibited the growth of *P. infestans*; this suggests that potato growers should utilize phosphate fertilizer supplemented with these micro-nutrients in order to increase the resistance of potato leaves to infection by *P. infestans*. Also, potato growers should not employ excessive amounts of nitrate fertilizer.

Glycoalkaloids do not appear to be toxic to common saprophytic and enteric bacteria. None of the microorganisms tested were inhibited by a natural mixture of glycoalkaloids. This apparent lack of toxicity may be due to the presence of a detoxifying enzyme or the inability of glycoalkaloid molecules to permeate the bacterial cell membrane.
LITERATURE CITED


Table 4. Analysis of variance of growth levels (g dry weight mycelium per 50 ml medium) of Phytophthora infestans race 1.2.4 corresponding to Figure 5

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<th>Source</th>
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</table>

LSD: 0.843 at 0.05 level.

Conclusion: Mycelium yields of Phytophthora infestans with 1.0-7.5% lactose, 2.5-7.5% maltose, 1.0-7.5% sucrose, and 1.0-2.5% galactose were significantly higher than the mycelium yields with French's unmodified medium or 5.0 and 7.5% galactose.

Table 5. Analysis of variance of growth levels (g dry weight mycelium per 50 ml medium) of Phytophthora infestans race 1.2.4 corresponding to Figure 6

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>14</td>
<td>0.79097</td>
<td>0.0594978</td>
<td>6.97</td>
<td>1.75</td>
</tr>
<tr>
<td>Error</td>
<td>99</td>
<td>0.80229</td>
<td>0.0081039</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>113</td>
<td>1.59326</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD: 0.916 at 0.05 level.

Conclusion: Mycelium yields of Phytophthora infestans with 9.0-12.0% maltose, 9.0-12.0% lactose, and 11.0-12.0% sucrose were significantly higher than the yields obtained with 8.0-10.0% sucrose, 8.0% maltose, and 8.0% lactose.
Table 6. Analysis of variance of growth levels (g dry weight mycelium per 50 ml medium) of *Phytophthora infestans* race 1.2.4 corresponding to Figure 7

<table>
<thead>
<tr>
<th>Source</th>
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<th>F</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>31</td>
<td>0.487241</td>
<td>0.0157174</td>
<td>47.81</td>
<td>1.65</td>
</tr>
<tr>
<td>Error</td>
<td>91</td>
<td>0.299202</td>
<td>0.0003287</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>122</td>
<td>0.786443</td>
<td>0.0021335</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD: 0.0260 at 0.05 level.

Conclusion: Mycelium yields of *Phytophthora infestans* with 7.5% galactose, 7.5% glucose, and 7.5% maltose were significantly higher than the yields obtained with 1.0-5.0% glucose, 1.0-5.0% maltose, 1.0-7.5% fructose, 1.0-5.0% galactose, 1.0-5.0% sucrose, 1.0-7.5% lactose, 1.0-7.5% arabinose, 1.0-7.5% mannitol, and French's unmodified medium.

Table 7. Analysis of variance of growth levels (g dry weight mycelium per 50 ml medium) of *Phytophthora infestans* race 1.2.4 corresponding to Figure 8

<table>
<thead>
<tr>
<th>Source</th>
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<th>MS</th>
<th>F</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>24</td>
<td>1.102384</td>
<td>0.0459326</td>
<td>12.12</td>
<td>1.57</td>
</tr>
<tr>
<td>Error</td>
<td>168</td>
<td>0.636501</td>
<td>0.0037886</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>192</td>
<td>1.738885</td>
<td>0.0075772</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD: 0.0617 at 0.05 level.

Conclusion: Mycelium yields of *Phytophthora infestans* with 12.0% glucose, 11.0 and 12.0% lactose were significantly higher than the mycelium yields obtained with 8.0-12.0% galactose, 8.0-12.0% maltose, 8.0-12.0% sucrose, 8.0-11.0% glucose, and 8.0-10.0% lactose.
Table 8. Analysis of variance of growth levels (g dry weight mycelium per 50 ml medium) of Phytophthora infestans race 1.2.4 corresponding to Figure 10

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
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<th>MS</th>
<th>F</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>47</td>
<td>3.275765</td>
<td>0.0696971</td>
<td>5.24</td>
<td>1.46</td>
</tr>
<tr>
<td>Error</td>
<td>96</td>
<td>1.276676</td>
<td>0.0132987</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>143</td>
<td>4.552441</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD: 0.1868 at 0.05 level.

Conclusion: Mycelium yields of Phytophthora infestans in media containing sodium salts were lower than the mycelium yields in the basal medium (control); however, the highest levels of growth in media containing sodium salts were not significantly lower than the yields in the control medium.

Table 9. Analysis of variance of growth level (g dry weight mycelium per 50 ml medium) of Phytophthora infestans race 1.2.4 corresponding to Figure 11

<table>
<thead>
<tr>
<th>Source</th>
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<th>MS</th>
<th>F</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>53</td>
<td>8.019339</td>
<td>0.1513082</td>
<td>35.66</td>
<td>1.43</td>
</tr>
<tr>
<td>Error</td>
<td>422</td>
<td>1.790659</td>
<td>0.0042432</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>475</td>
<td>9.809998</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD: 0.0610 at 0.05 level.

Conclusion: Mycelium yields of Phytophthora infestans were significantly higher in media containing 1.0-5.0 mg iron per liter than in the control medium. Growth of P. infestans was significantly inhibited by all concentrations of copper, cobalt, nickel, manganese, and by concentrations of calcium greater than 0.001 mg per liter.
Table 10. Analysis of variance of the percentage of samples that were positive for steroid glycoalkaloids corresponding to Figure 12

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
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<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>36</td>
<td>13176.491</td>
<td>366.01363</td>
<td>1.25</td>
<td>1.59</td>
</tr>
<tr>
<td>Error</td>
<td>74</td>
<td>21622.260</td>
<td>292.1927</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>110</td>
<td>34798.751</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD: None.
Conclusion: No significant differences between the percentage of samples positive for glycoalkaloids.

Table 11. Analysis of variance of glycoalkaloid content (mg total glycoalkaloids per 25 ml medium) in media in which Phytophthora infestans was cultured corresponding to Figure 13

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>36</td>
<td>1.9479004</td>
<td>0.0541083</td>
<td>1.73</td>
<td>1.59</td>
</tr>
<tr>
<td>Error</td>
<td>74</td>
<td>2.3092635</td>
<td>0.0312062</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>110</td>
<td>4.2571639</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD: 0.2876.
Conclusion: Upon substitution of sodium salts for the individual potassium and magnesium salts or the addition of sodium chloride to the basal medium, the average concentration of glycoalkaloids detected in the medium decreased significantly.
Table 12. Analysis of variance of the percentage of samples positive for glycoalkaloids corresponding to Figure 14

<table>
<thead>
<tr>
<th>Source</th>
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<th>SS</th>
<th>MS</th>
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<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>9</td>
<td>10824.280</td>
<td>1202.6977</td>
<td>4.88</td>
<td>2.90</td>
</tr>
<tr>
<td>Error</td>
<td>11</td>
<td>2172.872</td>
<td>246.6247</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>13537.152</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD: 34.6 at 0.05 level.

Conclusion: The percentage of samples positive for glycoalkaloids in the control medium and in medium containing 0.001 mg iron per 100 ml was significantly higher than the percentage of positive samples detected in media containing higher concentrations of ferrous iron.

Table 13. Analysis of variance of glycoalkaloid content (mg total glycoalkaloids per 25 ml medium) in media in which Phytophthora infestans race 1.2.4 was cultured corresponding to Figure 15

<table>
<thead>
<tr>
<th>Source</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>9</td>
<td>2.4023962</td>
<td>0.2669329</td>
<td>89.95</td>
<td>2.90</td>
</tr>
<tr>
<td>Error</td>
<td>11</td>
<td>0.0326436</td>
<td>0.0029676</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>2.4350398</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD: 0.1199 at 0.05 level.

Conclusion: The concentration of glycoalkaloids detected in the control medium was significantly higher than the level detected in media containing ferrous salts.
Table 14. Analysis of variance of the diameter of leaf lesions (mm) due to infection with *Phytophthora infestans* race 1.2.4 corresponding to Figure 16

<table>
<thead>
<tr>
<th>Source</th>
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<th>MS</th>
<th>F</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>14</td>
<td>1384.6832</td>
<td>98.9059</td>
<td>4.92</td>
<td>1.92</td>
</tr>
<tr>
<td>Error</td>
<td>47</td>
<td>944.6931</td>
<td>20.0998</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>2329.3763</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD: 6.38 at 0.05 level.

Conclusions: Potato plants receiving 20.0 or 25.0% potassium nitrate fertilizer were significantly susceptible to infection by *Phytophthora infestans* race 1.2.4 than potato plants receiving potassium dihydrogen phosphate or no fertilizer.
VITA

Melanie R. Maas

Candidate for the Degree of

Doctor of Philosophy

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Major Field: Biology/Ecology

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