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# Relationship Between Susceptibility to Pythium Seedling Blight and Pea Seed Exudates*

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# 豌豆種子分泌物과 Pythium에 依한 苗立枯病에 對한 感受性과의 關係

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## ABSTRACT

Seeds of pea cultivars susceptible to damping-off caused by *Pythium ultimum* produced sugars in their exudates in half the time required for resistant cultivars. Also, the amount of reducing sugars was much greater in seed exudates of susceptible than resistant cultivars. Raffinose, melibiose, sucrose, glucose, and fructose (but not maltose, xylose or ribose) were identified in both resistant and susceptible cultivars; however, more glucose and sucrose were detected in seeds from susceptible than from resistant cultivars. *Pythium ultimum* grew more profusely around seeds from, and in exudates of, susceptible than resistant cultivars.

## Introduction

That exudates are produced by seeds and roots of plants, as well as their influence on microorganisms in the vicinity of seeds or roots, is generally recognized (4, 7, 8). Many of these investigations treat the role of exudates on *Fusarium* species or other fungi on root diseases of agronomic and horticultural crops(7). Fewer efforts have been made to ascertain the role of seed exudates in damping-off caused by *Pythium* species.

Middleton (5) reported 148 hosts for *Pythium ultimum* and Escobar et al. (2) found that 42 commercial pea lines were susceptible to *P. ultimum*. Flentje and Saksena (4) found that exudates from pea cultivars that were susceptible to *P. ultimum* contained more soluble solutes and resulted in more mycelial growth than did exudates from less susceptible cultivars. Similar results were reported for *Pythium* damping-off of bean (6).

Experiments were done with pea cultivars resistant or susceptible to damping-off caused by *Pythium ultimum* for the relative kind and amounts of sugars present in the seed exudates and their rates of production, and, the relative effect of these exudates on mycelial growth.

## MATERIALS AND METHODS

Axenically produced seed exudate was obtained from genotypes of peas: *Pisum sativum* L. 'New Era', 'Minn. 378-A-3-W, and 'Green Giant, 447' (susceptible), and, 'Minn. 378-A-3-Bwr' and 'Minn. 353-1-G' (resistant). A single surface disinfected seed was put into an acid cleaned test tube containing 2 or 5*ml* of sterilized, deionized glassdistilled water. Five tubes were prepared per genotype and they were incubated for 24

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hours in the laboratory. Seeds were removed from tubes after 6 to 48 hours and checked for bacterial and fungal contamination by streaking the exudates from each tube on corn meal agar. The tubes were stored at 5 C during this time. The total volume of uncontaminated exudate and number of seeds were determined.

Quantitative amounts of reducing sugar in the exudate were determined by the phenol-sulfuric acid method of Dubois et al. (1). A 1m*l* portion of exudate was added to a colorimetric tube and 0.5m*l* of 5% aqueous phenol were added. The mixture was swirled and 2.5m*l* of concentrated sulfuric acid (reagent grade) was added to each tube. The exudate was diluted, when necessary, to 0.1, 0.04 or 0.02 volume with sterile glass-distilled water. A standard curve was made using glucose. After 10 miuntes, the absorbance of each tube was determined at 490 nm using a spectronic colorimeter against a sample blank. For the sample blank, phenol was omitted and replaced with sterile glass-distilled water because of the resistant genotypes were darker than these of the susceptible ones, and the pigments in the exudes affected absorbance appreciably.

Qualitative analysis of exudates for sugars was done with descending paper chromatography (3, 10) on whatman No. 1 filter paper (22×46.5cm) using the silver nitrate-sodiumhydroxide method (10). The solvent system used was N-propanol:ethyl acetate:water(7:1:2 v/v) (3). Chromatograms were spotted with 50 microliter samples of exudate, and irrigated with the solvent for 30 to 36 hours at 24° C. A standard solution of sugars was prepared individully at 1% each in 70% ethanol and a mixture was made to contain a 0.1% solution of each sugar. Ten microliters of the standard solution of sugars was spotted to give 1$\mu$g of each sugar per spot. The standard solution contained equal amounts of eight sugars: raffinose, melibiose, maltose, sucrose, glucose, fructose, xylose, and ribose. For detection of the separated sugars, air-dried chromatograms were dipped into a solution composed of 0.1m*l* of a standard aqueous silver nitrate solution in 20m*l* of acetone to which water was added dropwise to redissolve the silver nitrate. After drying the chromatograms, they were dipped into a solution of 0.5N sodium hydroxide in 70% ethanol, allowed to air dry, and then passed slowly through a 10% aqueous solution of sodium thiosulfate to remove the excess silver oxide background.

To ascertain silver nitrate sensitive substances in seed exudates, surface disinfected seeds were placed on filter paper, saturated with sterile, glass-distilled, deionized water, then set on glass plates 4cm apart on the papers. Seeds were removed from the filter paper at 6 hour intervals for up to 48 hours. Sugars were detected on airdried papers using the silver nitrate method of Schroth and Cook (6). The size and density of spots were considered indicators of the amount of sugar exuded. Comparisons were made also between seeds in which testae were intact and those in which testae were cut with a scalpel.

To study the effect of seed exudates on growth of *Pythium ultimum* Trow, individual surface disinfected seeds were placed in each of several acid cleaned test tubes each containing 5m*l* sterile, glassdistilled, deionized water, and incubated for 24 hours at 24 C. Ten uncontaminated, imbibed seeds of each line or cultivar were removed with their exudates and put into a sterile storage dish. Five ml of a suspension of hyphal fragments at 1000 fragments/ml were then axenically pipetted into each storage dish and the dishes were incubated at 24 C for several days. Each treatment consisted of three replicates and was repeated once. Mycelial growth was checked macroscopiclly 24, 48, 72, and 96 hours after incoulation. Treatments included exudate from each of the four genotypes separately with the seed and seed exudates, and a 1 : 1 mixture of exudate from susceptible and resistant genotypes (five seeds for each type).

In experiments with exudates from single genotypes only, the exudates from five uncontaminated seeds in test tubes (5 m*l*/seed), from which seeds were removed, were pipetted into a 125m*l* Erlenmeyer flask and inoculated with 2.5m*l* of a hyphal suspension (1,000 fragments/m*l*), and incubated at 24 C. Mycelial growth was checked macroscopically 24, 48, 72, and 96 hours after inoculation.

In dry weight determinations of mycelia, the seed exudates were prepared by soaking a single seed in each test tube containing 2m*l* of sterile, glass-distilled, deionized water, and uncontaminated exudates were removed after 48 hours and transferred to a 125m*l* Erlenmeyer flask(contents of 25 tubes added per flask). A disc of mycelium (5 mm diameter) from a 2-day-old

culture on a water agar medium was transferred to each flask and incubated for 7 days at 24 C. Dry weights were determined by oven drying mycelia for 24 hours at 60 C on a piece of aluminum foil.

## RESULTS

*Total sugar content of seed exudates.* By means of the silver nitrate method (6), in which size and density of spots on filter paper are considered indicators of sugar exuded from seeds, the susceptible genotypes generally exuded more silver nitrate-sensitive substances than resistant genotypes did. However, individual seeds of the resistant line Minn. 353-1-G sometimes exuded amounts of sugar comparable to that from the susceptible genotypes. Injuries to the testae increased the amount of sugars exuded from the susceptible genotypes but hardly at all from the resistant genotypes. Also, the susceptible genotypes exuded silver nitrate-sensitive substances more rapidly than resistant ones did. When seeds were placed on filter paper, it took 6 hours for susceptible genotypes and 12 or more hours for resistant genotypes to produce silver nitrate-sensitive substances.

Greater total amounts of redu ing sugars were present in exudates from susceptible genotypes than from resistant genotypes (Fig. 1). The amounts of reducing sugars in exudates from seeds of susceptible genotypes were greater than from resistant ones, beginning 12 hours after seeds were soaked in sterile, glass-distilled, deionized water. These results suggest that the amount of reducing sugars exuded from seeds during early stages of germination is related to resistance of peas to pre-emergence damping-off.

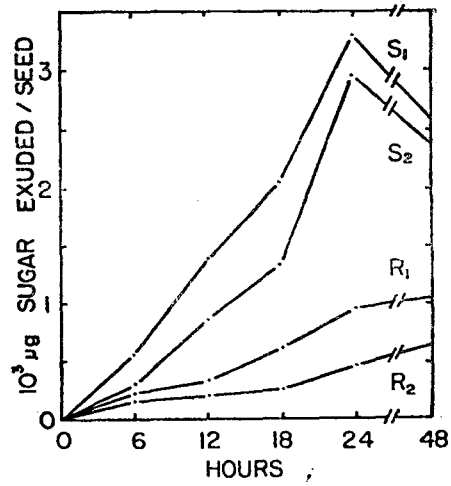


**Fig. 1.** Relative amounts of sugars exuded from seeds of four genotypes of peas, two of which are resistant ($R_1$ and $R_2$) and two susceptible ($S_1$ and $S_2$) to damping-off from *Pythium ultimum* based on three experiments and three replicates per experiment. $S_1$=Minn 378-A-3-W, $S_2$=New Era, $R_1$=Minn 353-1-G, and $R_2$=Minn 378-A-3-Bwr.

*Specific reducing sugars in exudates.* Chromatographic analysis of sugars present in seed exudates showed that there were no differences in the specific sugars in exudates of susceptible and resistant genotypes (Table 1). Raffinose, melibiose, sucrose, glucose and

**Table 1.** Relative amounts of sugar in seed exudates from peas resistant and susceptible to *Pythium ultimum* under axenic conditions

| Sugar | Standard[a] | New Era[b] | Minn. 378-A-3-W[b] | Minn.378-A-3-Bwr[c] | Minn. 353-1-G[c] |
|---|---|---|---|---|---|
| Raffinose | ++ | +++ | +++ | ++ | ++ |
| Melibiose | ++ | +++ | +++ | ++ | ++ |
| Maltose | ++ | − | − | − | − |
| Surose | ++ | ++++ | ++++ | ++ | +++ |
| Glucose | ++ | ++++ | ++++ | ++ | +++ |
| Fructose | ++ | +++ | +++ | ++ | ++ |
| Xylose | ++ | − | − | − | − |
| Ribose | ++ | − | − | − | − |

a Chromatograms were spotted with 10 microliters of a standard solution of sugars containing 1$\mu$g of each/10 microliters. Fifty microliters of each sample were spotted.

b Susceptible to *Pythium ultimum*

c Resistant to *Pythium ultimum*

fructose, but not maltose, xylose were identified in the exudates.

More glucose and sucrose were detected in exudates from susceptible than resistant genotypes. Increases were noted for the remaining three sugars but not to the same degree. Considerable amounts of other unidentified silver nitrate-po itive substances were present in susceptible genotype exudates that were not present in resistant genotype exudates. Also, there were apparently large quantities of unknown materials on spots from the resistant genotypes not present in spots from susceptible genotypes; however, none of these substances were identified.

*Effect of seed exudates on growth of Pythium ultimum.* Mycelial growth was more profuse near seeds of the susceptible genotypes than near seeds of the resistant ones after 4 days (Table 2). Differences were especially obvious within 24 to 48 hours of inoculation, in which there was no mycelial growth in seed exudates of resistant genotypes. By mixing exudates of seeds from resistant and susceptible genotypes, mycelial growth was apparent after 48 but not at 24 hours. Fungus growth was initiated around the seeds which were left in the solution exudate.

**Table 2.** Effect of seed exudates of pea genotypes resistant and susceptible to *Pythium ultimum* on mycelial growth

| Source of exuate | Host reaction[a] | Relative mycelial growth after hour indicated[b] | | | |
|---|---|---|---|---|---|
| | | 24 | 48 | 72 | 96 |
| *Seeds and seed exudates* | | | | | |
| New Era | S | + | ++ | +++ | ++++ |
| Minn. 378-A-3-W | S | + | ++ | +++ | ++++ |
| Minn. 353-1-G | R | − | − | + | ++ |
| Minn. 378-A-3-Bwr | R | − | − | + | ++ |
| +New Era | | − | + | ++ | ++ |
| Minn. 378−A-3-W | | | | | |
| +Minn. 353-1-G | | − | + | ++ | ++ |
| *Seed-free exudates* | | | | | |
| New Era | S | + | ++ | ++++ | ++++ |
| Minn. 378-A-3-W | S | + | ++ | ++++ | ++++ |
| Minn. 353-1-G | R | − | − | + | ++ |
| Minn. 378-A-3-Bwr | R | − | − | + | ++ |

a Based on three replicates of two different experiments;−indicates no hyphal growth and+indicates growth.
bR=resistant and S=susceptible to *Pythium ultimum.*

**Table 3.** Effect of seed exudates on dry weight of *Pythium ultimum*

| Source of exudate | Host reaction | Mycelial dry weight (μg)[a] |
|---|---|---|
| New Era | S | 4,850±1,300 |
| 447 | S | 5,130±1,580 |
| Minn. 378-A-3-W | S | 4,810±1,240 |
| Minn. 353-1-G | R | 1,610± 880 |
| Minn. 378-A-3-Bwr | R | 1,230± 650 |
| Control[b] | | 110± 60 |

a Each datum represents the average of three replicates and three different experiments
b Sterile glass-distilled, deionized water.

In a second experiment, the seeds were removed from the solution and the fungus grew in the seed-free exudate. The results were similar (Table 3 vs. Table 2). The dry weights of mycelium were considerably higher when the fungus grew in seed exudates from susceptible genotypes than from resistant ones (Table 4). The seed exudate was sterilized either by filtering it through a Millipore filter or by autoclaving it;neither procedure affected the exudates from any genotype.

## DISCUSSION

There seems to be a general correlation between

amount of seed exudation and susceptibility to pre-emergence damping-off due to *Pythium* species or other fungi. Schroth and Cook (6) noted that the greatest seed exudation occurred from cultivars most susceptible to damping-off in bean. This held for peas in the present investigation in which sugars in the exudate were in greater quantity from cultivars susceptible to *Pythium ultimum*. Glucose and sucrose appeared to be the most important sugars influencing *Pythium* growth. Cultivars that produced more of these two sugars were more susceptible to pre-emergence damping-off. Thus resistance to damping-off may be developed by selection for less sugar in the exudate.

Short and Lacy(8) noted that favored high amounts of nutrient exudation generally corresponded with conditions that favored high percentages of spore germination around seeds and high amounts of seed decay. They also reported that soaking seeds of pea in water prior to planting reduced incidence of seed and seedling rot presumably due to the removal of most of the exudate (9). The preponderance of carbohydrates was exuded at 22 or 30 C during the first 18 hours' incubation, but at 10 C significant exudation occurred for about 48 hours (8). This means that exudates are influenced by temperature and moisture at planting, as well as by genetic factors in the host.

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## 摘　　要


*Pythium ultimum*에 依한 苗立枯病에 感受性인 豌豆品種의 種子는 抵抗性品種의 種子보다 多量의 還元糖이 種子分泌物內에 包含되었으며 分泌速度도 感受性인 豌豆品種의 種子에서 더 빨랐다. 苗立枯病에 抵抗性인 豌豆品種의 種子와 感受性인 品種의 種子分泌物에서 모두 Raffinose, Meilbiose, Sucrose, Glucose 및 Fructose가 確認되었으나 Glucose와 Sucrose는 感受性品種의 種子分泌物에서 더많이 검출되었다. *Pythium ultimum*菌은 感受性인 種子및 種子分泌物에서 더잘자랐다.