HOST-PARASITE INTERACTIONS OF ENZYME-SEPARATED CELLS OF SOYBEAN LEAVES AND XANTHOMONAS PHASEOLI VAR. SOJENSIS

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Major professor

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ABSTRACT

HOST-PARASITE INTERACTIONS OF ENZYME-SEPARATED CELLS OF SOYBEAN LEAVES AND XANTHOMONAS PHASEOLI VAR. SOJENSIS

by Joseph A. Ignatoski

Host-parasite interactions were studied by examination of interactions between enzyme-separated plant cells and bacteria. Interactions were defined to be mutual influences of enzyme-separated cells and bacteria on each other. A basic premise of the work was that the mutual influences are host-parasite interactions if they are part of the mechanism that makes the bacteria parasitic.

Interactions were detected by measuring the changes in growth rate of parasitic bacteria (X. phaseoli var. sojensis) when incubated with enzyme-separated host cells (soybean cvs. Blackhawk and Lee). The growth rate of the bacteria was measured by the dilution pour plate technique. Counts were made after 72 hrs at 23° C. Soybean cells were obtained from leaves by enzymatic digestion of the middle lamella. Growth rates of a saprophyte (S. marcescens) when incubated with enzyme-separated soybean cells were also measured. The incubation medium was a

modified White's medium specifically designed for soybean tissue culture.

Interactions were observed between bacteria and the enzyme-separated soybean cells. The interactions were detected by an increase in the rate of bacterial growth. The initiation and rate of bacterial growth depended on the concentrations of bacteria and enzyme-separated soybean cells.

Differences in the interactions of enzyme-separated cells of the resistant (cv. Lee) and susceptible hosts (cv. Blackhawk) were observed when incubated with the parasite. They were different because the numbers of bacteria and host cells needed to produce the interaction were different. One hundred times as many bacteria, (X. phaseoli var. sojensis) and enzyme-separated cells of the resistant host (soybean, cv. Lee) were needed to produce the same growth of the bacteria as they exhibited when incubated with cells of the susceptible host (soybean cv. Blackhawk). The susceptible host-parasite interaction products were detected in filtrates of media which previously contained the parasite and the cells of the susceptible host for a period of 6 hrs or more. The resistant host-parasite interaction products were detected in filtrates of media which previously contained the parasite and cells of the resistant host for a period of 8 hrs or more.

The interaction product(s) were different in constituency of concentration because filtrates containing the interaction product(s) of the resistant host-parasite combination took a longer period of time to stimulate the parasite to grow. The parasite (X. phaseoli var. sojensis) did not grow when incubated with cells of a non host (N. tabacum). Another parasite (Pseudomonas angulata) did grow when incubated with enzyme-separated cells of a susceptible hose (soybean cv. Lee or N. tabacum).

No differences in interaction were observed with either soybean cv. and the saprophyte. The number of bacteria and enzyme-separated soybean cells needed to produce the interaction were the same. The interaction product(s) appeared to be produced at the same time and stimulated the saprophyte to grow at the same rate.

Differences in interactions of parasitic and saprophytic bacteria were observed when the bacteria were incubated with enzyme-separated cells of soybean (cvs. Blackhawk and Lee). The parasite-host cell combinations required ten to one-thousand times as many parasite and host cells to produce the interaction than the saprophyte-soybean cell combinations. The enzyme-separated cells of the susceptible host induced the parasite to produce product(s) which caused the host cells to release substances which stimulated the parasite to grow. The saprophyte, on the other hand, caused the enzyme-separated soybean cells to

release or produce substances which stimulated the growth of the saprophyte.

The differences in the interaction of a parasite with cells of a resistant and susceptible cv. appear to reflect differences in the cvs. These differences may reflect host-parasite interactions because: (1) no difference in the cvs. was observed in their interaction with the saprophyte, and (2) the interactions of these two cvs. with the saprophyte are different than either cv. with the parasite.

HOST-PARASITE INTERACTIONS OF ENZYME-SEPARATED CELLS OF SOYBEAN LEAVES AND *XANTHOMONAS PHASEOLI* VAR. *SOJENSIS*

By

Joseph A. Ignatoski

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To my wife

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INTRODUCTION

My purpose in this work was to study host-parasite interactions. The specific plant materials chosen for study were enzyme-separated cells of soybean and tobacco leaves, prepared by pectinase digestion of the middle lamella. The parasites were bacteria that invade the leaves of these plants. Specifically, they were Xanthomonas phaseoli var. sojensis and Pseudomonas angulata. Common saprophytes, Serratia marcescens and Escherichia coli, were also used to detect interactions with plant cells. The basic assumptions of the work are that there are mutual influences of the parasite and host on each other--that the products of the host affect the parasite and that the products of the parasite affect the host. There is much circumstantial and some direct evidence that such interactions occur (3, 14, 37, 45).

Interaction is a key word. Inter- is a preposition denoting between and action is a noun meaning act or process of producing an effect. Thus, interaction is mutual action or influence. Interactions of plant cells and bacteria are mutual influences of plant cells and bacteria on each other. If these mutual influences, or interactions, are part of the mechanism which makes the

bacteria parasites and the plant cells hosts, then the mutual influences are host-parasite interactions.

My experiments were designed to detect host-parasite interactions and the time of their occurrence. Bioassays involving growth of parasitic and saprophytic bacteria were chosen as a method of detecting products of the host-parasite interactions. Bacterial growth rates should indicate the presence of stimulatory or inhibitory products of the host-parasite interactions.

Bacterial leaf diseases were chosen because the causal bacteria are unicellular and therefore quantitative growth measurements in short time periods are possible. The bacteria are extracellular parasites and pathogens. They enter the leaf through stomates, other natural openings, and wounds and lodge in a film of moisture. Host-parasite interactions occur in this film of moisture which covers the surface of the cells of the substomatal chamber and other intercellular spaces. On the other hand, interactions of an intracellular parasite occur within the host cells. The products of such interactions cannot be isolated without host cell disruption which terminates the host-parasite interactions and mixes the cellular contents with the interaction products.

Enzyme-separated cells were used because the extracellular relationship of the bacteria and host cells is maintained, but with certain advantages. All of the bacteria and enzyme-separated cells can engage in the

host-parasite interactions. In the intact plant, the number of host cells engaging in the interactions with the parasite are few. Only the cells about the foci of infection are involved. Products of interactions under these conditions are most likely in low concentrations and are, therefore, difficult to detect.

LITERATURE REVIEW

Hosts can affect their parasites by the production of inhibitory or stimulating substances (28, 44). Root exudates are stimulatory substances which attract zoospores (58), promote germination of soil microorganisms (42), and appear to influence the site of infection (13). Phenolic compounds in the outer scales of onions are inhibitory substances which make colored onions resistant to onion smudge (52, 53). Plants also produce substances called phytoalexins, in response to parasites (14).

Parasites can affect their hosts by the production of toxins (37), enzymes (3), and growth regulators (45). These substances can cause necrosis (43), chlorosis (4, 5), maceration (7) and abnormal growth (48).

The interactions of host and parasite may also be affected by the numbers of host and parasite cells interacting. Tomiyama et al. (50) have shown that slices of potato tubers which are resistant to late blight will react as do the slices of susceptible tubers when the slices are less than 10 cells in average thickness. Scharen found that Xanthomonas phaseoli (E. F. Smith) Stevens can induce symptoms in resistant cultivars when introduced into the leaves in high numbers (41).

Numbers of bacteria and their rate of increase have been examined in intact leaves of plants. Scharen (41) found that X. phaseoli attained higher numbers in a susceptible cultivar of bean than in a resistant one. The rate of multiplication was equal in both cultivars up to the third day, after which the bacteria began to decline in the resistant one. Other workers (2, 10, 11, 16, 46) have demonstrated similar increases in the number of bacteria in resistant as well as susceptible plant tissues with X. phaseoli, X. phaseoli var. sojensis (Hedges) Burk and Starr, X. vesicatoria (Doidge) Dowson, Pseudomonas glycinea Coerper, and P. lachrymans (Smith and Bryan) Carsner. None of these workers collected data during the first 24 hours or described differences in growth rates during this period. Large differences in growth rates of bacteria in the susceptible and resistant cultivars are evident, however, if their data are plotted on a semi-logarithmic scale. No appreciable differences in growth rates are evident after this period.

Data of Klement et al. (29) show differences between the growth rates during the first 24 hours of P. tabaci (Wolf and Foster) Stevens, a parasite of tobacco, and P. syringae Van Hall, a nonparasite of tobacco. The non-parasite grew even faster than the parasite in tobacco leaves after the initial lag period but did not attain as high a number regardless of the initial number of bacteria in the inoculum. The authors did not indicate

that the rates of growth are different but concluded that saprophytes cannot grow in plant tissues.

In an earlier publication (30), Klement et al. found that P. aeruginosa (Schroeter) Migula, another saprophyte grew in tissues of intact bean pods. The rate of multiplication of this saprophyte during the first 24 hours was found to exceed that of three parasites of bean: P. phaseolicola (Burkholder) Dowson, X. phaseoli, and X. phaseoli var. fuscans (Burkholder) Starr and Burkholder. Additional data on P. aeruginosa (31) indicate a lag of 6 says before begins. Even though the data of Klement et al. and others (2, 10, 11, 16, 41, 46) are confusing and conflicting, three observations can be made. Parasitic bacteria can multiply in resistant and nonhost plant tissues; parasitic bacteria in high numbers, can induce symptoms in nonhost plant tissues; and, important events concerning multiplication are occurring during the first 24 hours. Histological studies also show that there are differences in the reactions of susceptible and resistant plant tissues during the first 24 hours (20, 36, 40, 46, 49, 54).

One approach to studying multiplication rates of parasitic and nonparasitic bacteria in plant tissues is the utilization of enzyme-separated cells prepared by digestion of the middle lamella with pectinase.

The technique of separating plant cells from intact tissues by enzymatic digestion of the middle lamella

was first described by Emsweller and Stuart in 1944 (18). They used this technique to facilitate observation of meiotic configurations of chromosomes. Later, Chayen used a similar method to study mitotic configurations in cells of root tips (12). Electron microscope observations of enzyme-separated cells showed that the cell walls remained intact and without holes (56).

Enzyme-separated cell systems have been used in the study of ion uptake (27), amino acid synthesis (38), protein synthesis (39) and virus multiplication (57).

MATERIALS AND METHODS

Bacteria

The following bacteria were used in this study:

1. X. phaseoli var. sojensis, a pathogen of soybean (24)
2. Pseudomonas angulata (Fromme and Murray) Holland, a pathogen of soybean and tobacco (1, 21)
3. Serratia marcescens Bizo, a saprophyte found in soil (5)
4. Escherichia coli (Migula) Castellani and Chalmers, a saprophyte found in soil (5).

The two bacterial pathogens were chosen because they are similar to each other (9, 25), stable (10), have limited host ranges (6), and do not produce known secondary toxins (4, 5). The two saprophytes were chosen because they are common, easily identified, and are gram negative rods, as are the pathogens (6). Each isolate was transferred every 24 hours on nutrient agar. The bacteria under such conditions are in a log phase of growth and should be in the same physiological state from one experiment to another. A culture grown for 24 hours contains 10^8 cells/ml when suspended in 10 ml of incubation medium.

The appropriate dilutions were then made depending on the experiment. Pathogenicity tests were made before and after each series of experiments. In no case was a loss of pathogenicity observed with either pathogen.

Plant Materials

Greenhouse grown tobacco (Nicotiana tabacum L.) and soybeans (Glycine max (L.) Merrill) were used. Soybean cvs. Blackhawk and Lee were chosen because they are susceptible and highly resistant, respectively, to X. phaseoli var. sojensis (22, 26). The resistance of cv. Lee is controlled by a single recessive gene (19, 22, 26). Both cultivars are susceptible to P. angulata, and develop lesions after inoculation. N. tabacum is also susceptible to P. angulata (16), but is a nonhost for X. phaseoli var. sojensis (5). No symptoms were produced when the saprophytes, S. marcescens and E. coli, were introduced into tobacco or soybean leaves.

Enzymatic Separation of Leaf Cells

Enzyme-separated leaf cells were obtained by digestion of the middle lamella with pectinase as described by Jyung et al. (27). The separation mixture contained the following: glycerol, 0.2%; pectinase, 0.4% (rather than 0.2% as originally described); peptone, 0.2%; sucrose, 0.1 M; ethylenediaminetetraacetic acid (EDTA) (pH 6.4), 0.02 M; Tris-maleate (pH 6.4), 0.02 M; K_3-citrate, 0.01 M; and Na_2-succinate, 0.01 M. Pectinase

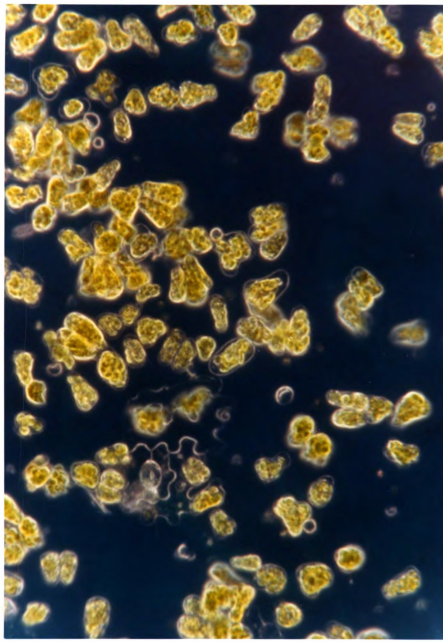
was obtained from the Sigma Chemical Company, St. Louis, Missouri. Bacteriological peptone was obtained from Matheson Coleman and Bell Company, Cincinnati, Ohio. When large numbers of the enzyme-separated cells (10^6/ml) were needed in volumes of 500 ml or more, the yield was doubled by macerating the remaining leaf tissue in a Waring blender for 30 seconds in an ice cold solution of 0.35 M sucrose plus phosphate buffer (pH 6.4). This was done because many enzyme-separated cells were trapped between the upper and lower cuticle of the leaf.

The separation procedure was the same as that described by Zaitlin (57) with one exception. When the leaves were macerated by the Waring blender, the foam that formed contained 90% of the enzyme-separated cells. This portion was suspended in the cold solution and washed by Zaitlin's method. The final suspension of enzyme-separated cells contained a mixture of spongy mesophyll and palisade cells along with a few guard cells (Fig. 1).

Enzyme-separated cells were viable, and bacterial and fungal contamination was negligible. A solution that contained 5000 mg (dry wt) of enzyme-separated cells/ml contained less than one fungal and bacterial contaminant/ml. The enzyme-separated cells were dark green and apparently healthy. Slight plasmolysis was observed in 4% of the cells at 33 hours after harvest. The enzyme-separated cells respired 20 µl/hr/mg dry weight (15) and 97% were found to be viable by means of a methylene blue stain (51).

FIGURE 1

Enzyme-separated cells of leaves of soybean, cv. Blackhawk.



This figure did not change after the cells were stored for 12 hours in a solution of 0.35 M sucrose-phosphate buffer (pH 6.4) at 0° C. or after 12 hours in the incubation medium at 23 ± 2° C.

Twelve hours after harvest the enzyme-separated cells were spun down at 800 g for 15 minutes and resuspended, in the appropriate concentration, in the incubation medium (28). The incubation medium was specifically designed for soybean tissue culture by Miller (34). The medium contained the following (mg/liter): KNO_3, 1000; NH_4NO_3, 1000; $Ca(NO_3)_2 \cdot 4H_2O$, 500; KCl, 65; $MnSO_4 \cdot 4H_2O$, 14; NaFe EDTA, 13.2; $ZnSO_4 \cdot 7H_2O$, 3.8; H_3BO_3, 1.6; nicotinic acid, 0.5; pyridoxine · HCl, 0.1; thiamine · HCl, 0.1; glycine, 0.3; kinetin, 0.5; 3-indolylacetic acid, 5; sucrose, 30,000; phosphate buffer (6.4) and KI, 0.8 (35). The medium was stored at 0° C.

Preparation of Suspensions of Enzyme-Separated Cells and Bacteria

Suspensions of separated-cells were adjusted to desired concentrations by measuring the density with a Spectronic 20 Colorimeter. A suspension of enzyme-separated cells of 500 µg/ml (dry wt) was found to contain 10^5 cells/ml and gave 40% light transmission in the colorimeter at a wavelength of 490 mµ.

Incubation medium and bacterial suspensions for 3 replications of an experiment was prepared in a single

batch to reduce variability. The desired number of bacteria was suspended in 150 ml of the media. The desired number of enzyme-separated cells were spun down at 800 g for 15 min. and the supernatant discarded. The enzyme-separated cells were then resuspended in the inoculated media. This mixture was divided into three 50 ml portions and distributed to 250-ml flasks. These were incubated at 23 ± 2° C.

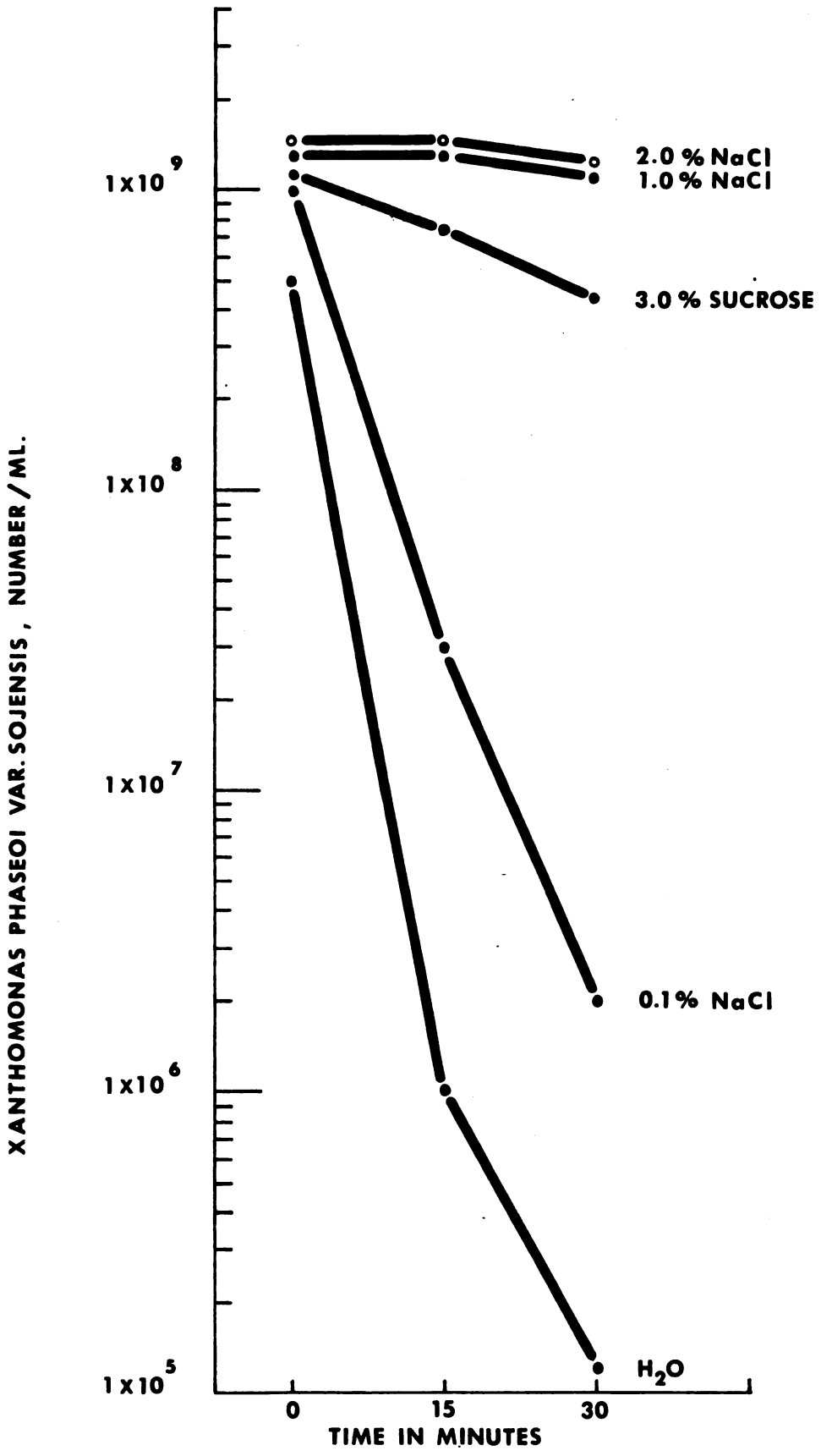
Census of Bacterial Populations

Numbers of viable bacteria were determined by counting colonies resulting from plating dilutions of samples in nutrient agar. Dilutions were made in 1% sodium chloride because X. phaseoli var. sojensis was found to die rapidly in water and in solutions of 0.1% sodium chloride and 3% sucrose (Fig. 2). Thus, data obtained by other workers (10) using water as a diluting medium for this and other fragile bacteria are open to question (2, 9, 16. 29, 41). Studies on growth rates of bacteria were conducted using 10^2 bacteria/ml when possible. This concentration of bacteria eliminates any error due to dilutions because no dilutions are required.

Nutrient agar (0.6%) was prepared by making appropriate mixtures of nutrient broth (Difco) and agar (Difco). X. phaseoli var. sojensis was killed by exposure to 48-49° C. for 10 minutes. Agar at 0.6% gels at room temperature but can be held as a liquid at 40° C.

FIGURE 2

Survival of Xanthomonas phaseoli var. sojensis in water, 3% sucrose and 0.1, 1.0, and 2.0% sodium chloride.


XANTHOMONAS PHASEOI VAR. SOJENSIS, NUMBER / ML.
1x10^9
1x10^8
1x10^7
1x10^6
1x10^5
2.0 % NaCl
1.0 % NaCl
3.0 % SUCROSE
0.1% NaCl
H2O
0
15
30
TIME IN MINUTES

Graphs

Each point on a graph represents the average value of three replications. In addition each experiment was performed at least twice to confirm results.

RESULTS

Growth Rates of Bacterial Parasites When Incubated with Enzyme-Separated Cells of Susceptible and Resistant Plants

The number of bacteria in contact with host tissues can affect the response of the host to the bacteria (41). Likewise, the number of host cells exposed to a parasite can affect the response of the parasite to the host (50). I did the following experiments to determine the effect of the concentration of bacteria and enzyme-separated cells on the growth rate of the bacteria. The parasite (*X. phaseoli* var. *sojensis*), in concentrations from 10^2 to 10^6 bacteria/ml, was suspended with enzyme-separated cells, in concentrations from 5 to 5,000 µg/ml. The cvs. Blackhawk (susceptible) and Lee (resistant) were used.

The growth rate of the parasite increased with an increase in the concentration of bacteria and with an increase in the concentration of enzyme-separated cells of the susceptible cv. (Fig. 3). When incubated with the resistant cv., the parasite was observed to increase in number only when the highest concentrations of the parasite (10^6 bacteria/ml) and host cells (5,000 µg/ml) were used (Fig. 4). These experiments were performed three times with the same results. The parasite did not

FIGURE 3

Growth of Xanthomonas phaseoli var. sojensis when incubated with enzyme-separated cells of a susceptible cv. of soybean, Blackhawk. Various concentrations of bacteria and host cells were used.

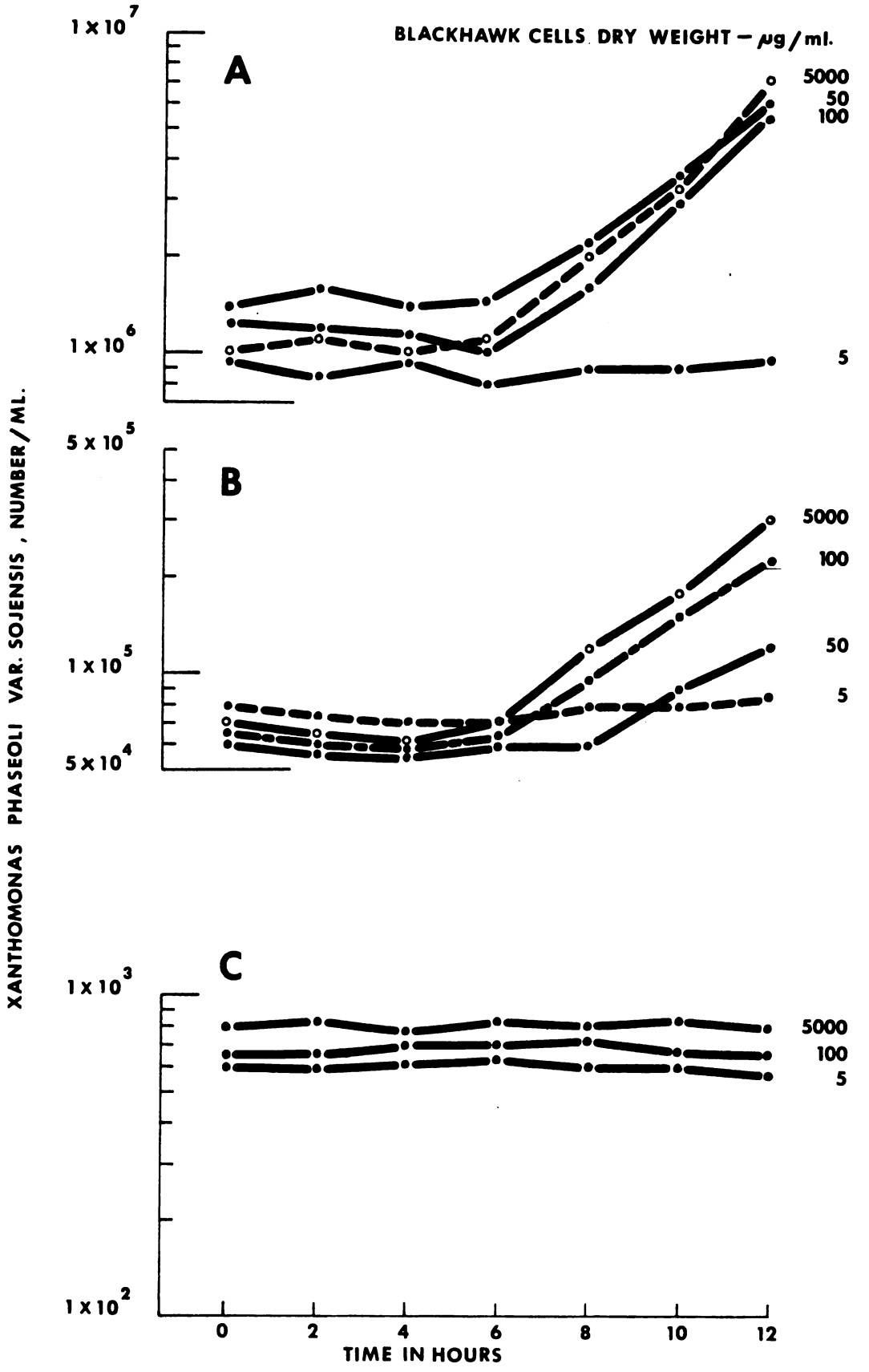
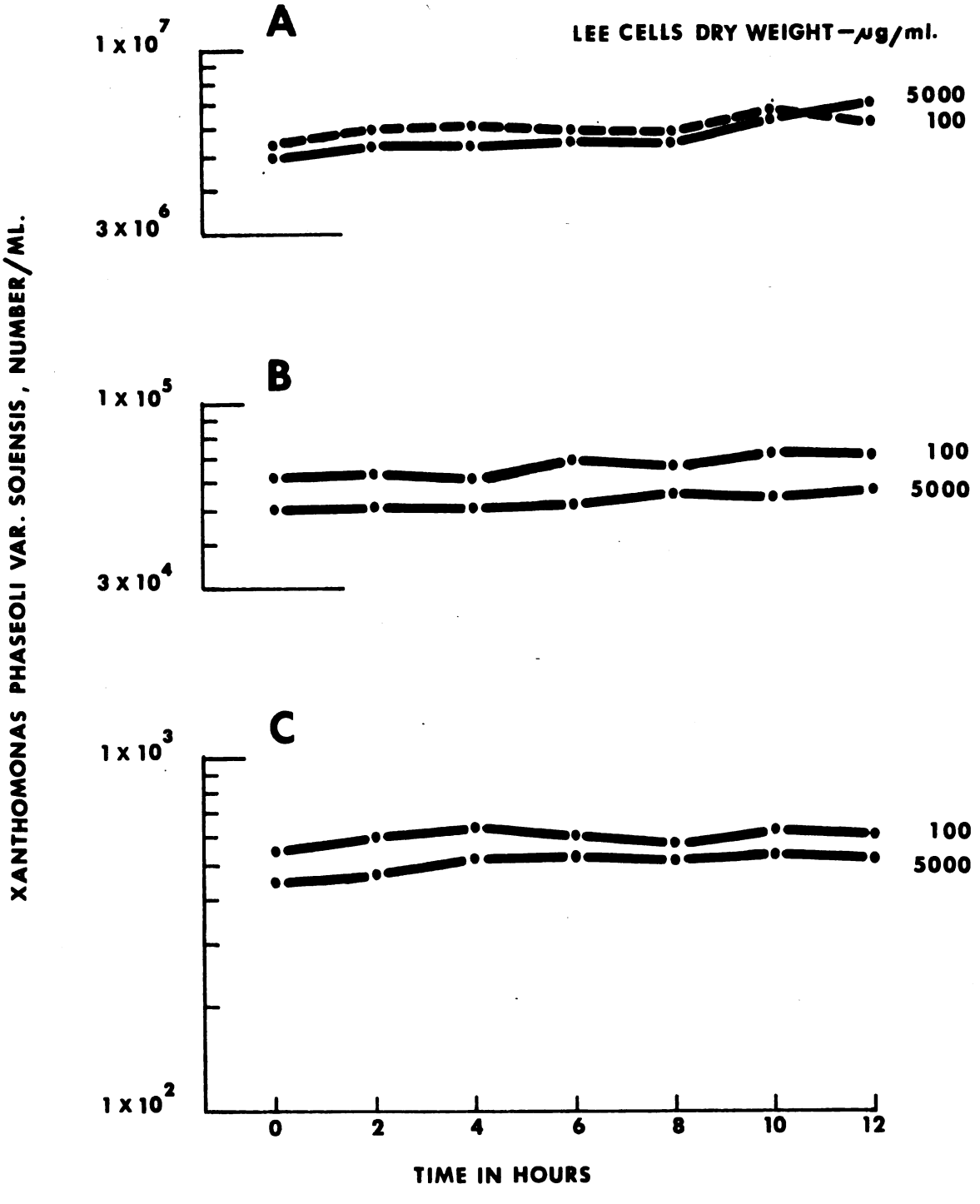

BLACKHAWK CELLS DRY WEIGHT – µg/ml.
A
B
C
5000
100
50
5
1 x 10^7
1 x 10^6
5 x 10^5
1 x 10^5
5 x 10^4
1 x 10^3
1 x 10^2
0
2
4
6
8
10
12
TIME IN HOURS
XANTHOMONAS PHASEOLI VAR. SOJENSIS, NUMBER/ML.

FIGURE 4

Growth of Xanthomonas phaseoli var. sojensis when incubated with enzyme-separated cells of a resistant cv. of soybean, Lee. Various concentrations of bacteria and host cells were used.


A
LEE CELLS DRY WEIGHT—µg/ml.
1 x 10^7
3 x 10^6
5000
100
B
1 x 10^5
3 x 10^4
100
5000
C
1 x 10^3
1 x 10^2
100
5000
0
2
4
6
8
10
12
TIME IN HOURS
XANTHOMONAS PHASEOLI VAR. SOJENSIS, NUMBER/ML.

grow in the medium alone (Figs., 5D, 6D). The pH (6.4) remained constant throughout all of the experiments and the numbers of dead host cells did not change significantly during any experiment.

The growth of the parasite when incubated with enzyme-separated cells of the susceptible cv. (Fig. 3), or with the resistant cv. (Fig. 4), would appear to be due to substance(s) released or produced by the host cells, because the parasite did not grow in the media alone. Because an increase in the number of parasite and host cells of either cv. determine this growth response, it would seem that the parasite causes the host cells to release or produce the stimulatory substance(s). These stimulatory substances, then, are products of interaction of the parasite and the host cells.

Higher concentrations of enzyme-separated cells of the resistant cv. than of the susceptible cv. were needed to induce growth of the parasite. This difference may or may not be related to the difference in susceptibility of the cv. to the parasite.

Growth Rates of X. phaseoli var. sojensis in Filtrates

In order to determine whether or not the stimulatory substances were the result of interaction between the parasite and host cells, the following experiments were conducted. The parasite (X. phaseoli var. sojensis) was grown in filtrates from media which previously contained

FIGURE 5

(A - C) Growth of Xanthomonas phaseoli var. sojensis in filtrates of media in which were incubated for 0.2 to 12 hrs, (A) a mixture of enzyme-separated cells (5,000 µg/ml) of the susceptible cv. of soybean (Blackhawk) and X. phaseoli var. sojensis (10^6 bacteria/ml); (B) only enzyme-separated cells (5,000 µg/ml) of the susceptible cv.; and, (C) only X. phaseoli var. sojensis (10^6/ml).

(D) Growth of X. phaseoli var. sojensis in incubation medium alone.

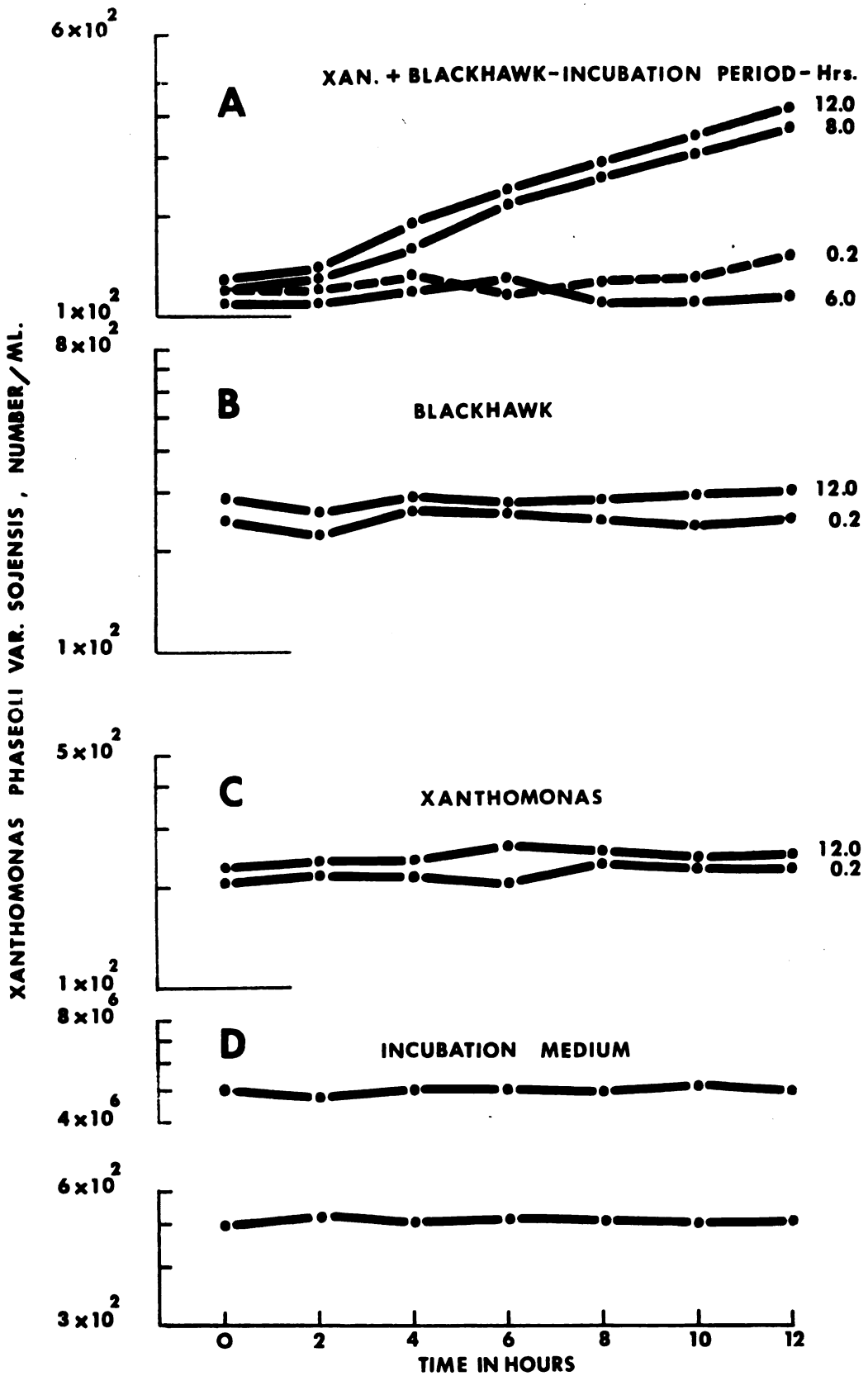
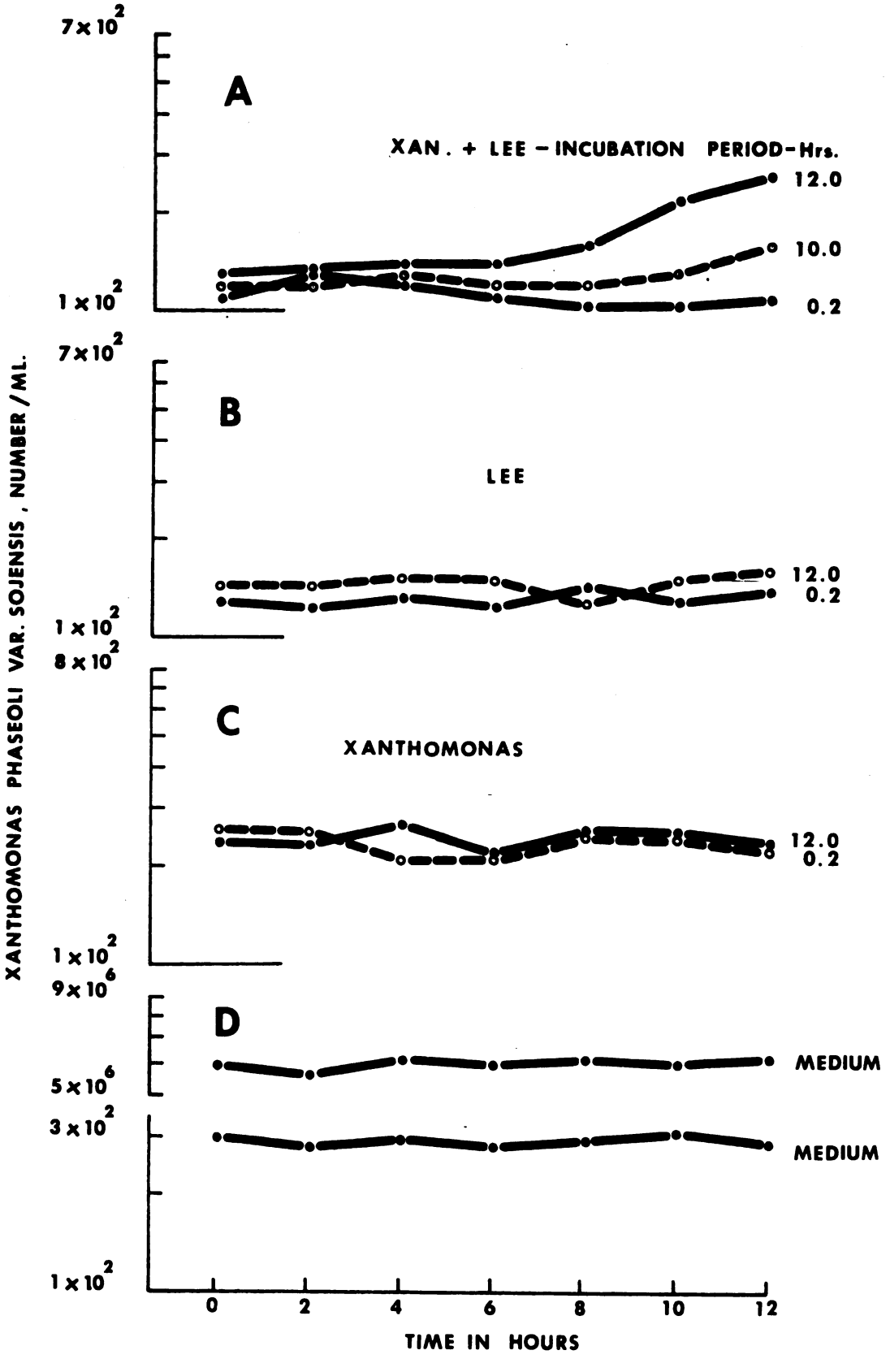

XANTHOMONAS PHASEOLI VAR. SOJENSIS, NUMBER/ML.
6x10^2
1x10^2
8x10^2
1x10^2
5x10^2
1x10^2
8x10^6
4x10^6
6x10^2
3x10^2
A
XAN. + BLACKHAWK-INCUBATION PERIOD-Hrs.
12.0
8.0
0.2
6.0
B
BLACKHAWK
12.0
0.2
C
XANTHOMONAS
12.0
0.2
D
INCUBATION MEDIUM
0
2
4
6
8
10
12
TIME IN HOURS

FIGURE 6

(A - C) Growth of Xanthomonas phaseoli var. sojensis in filtrates of media in which were incubated for 0.2 to 12 hrs, (A) a mixture of enzyme-separated cells (5,000 µg/ml) of the resistant cv. of soybean (Lee) and X. phaseoli var. sojensis (10^6 bacterial/ml); (B) only enzyme-separated cells (5,000 µg/ml) of the resistant cv.; and, (C) only X. phaseoli var. sojensis (10^6/ml).

(D) Growth of X. phaseoli var. sojensis in the incubation medium alone.


A
XAN. + LEE – INCUBATION PERIOD–Hrs.
12.0
10.0
0.2
B
LEE
12.0
0.2
C
XANTHOMONAS
12.0
0.2
D
MEDIUM
MEDIUM
7x10^2
1x10^2
8x10^2
9x10^6
5x10^6
3x10^2
0
2
4
6
8
10
12
TIME IN HOURS
XANTHOMONAS PHASEOLI VAR. SOJENSIS, NUMBER/ML.

either: (1) the parasite only (10^6 bacteria/ml), (2) the enzyme-separated host cells only (5,000 µg/ml), or (3) mixtures of the two. Filtrates of the incubated cultures were collected and sterilized by filtration at two hr intervals beginning at 0.2 hrs and ending at 12 hrs.

The parasite did not grow in any of the filtrates from media which previously contained only the parasite (Fig. 5-C, 6-C) or only the enzyme-separated cells of either the susceptible (Fig. 5-B) or the resistant cv. (Fig. 6-B).

Growth of the parasite was observed only in filtrates of media which previously contained mixtures of parasite and host cells (Figs. 5-A, 6-A). Increases in bacterial growth were observed in filtrates collected after 8 hrs or more of incubation of the parasite with cells of the susceptible cv. and after 10 hrs or more of incubation with cells of the resistant cv. There was a lag of 2 to 4 hrs before the growth increased in the former case (susceptible cells) and a lag of 6 to 8 hrs in the latter (resistant cells). Clearly, the increase in growth rate is due to stimulatory substance(s) released or produced by the host cells in response to the presence of the parasite. These stimulatory substances are products of interaction.

Release or production of bacterial stimulated substances occurred before the eighth hour in the susceptible

host-parasite combination and before the tenth hour in the resistant host-parasite combination. Stimulatory interaction product(s) were first detected in filtrates collected from samples incubated for those time periods (Figs. 5-A, 6-A).

To determine the duration of the lag period in growth of the parasite in filtrates that contain stimulatory interaction product(s), the following experiments were conducted.

Bacteria were incubated for 10 hrs in filtrates of media that previously contained mixtures of bacteria (10^6/ml) and enzyme-separated cells (5,000 µg/ml) for 12 hrs. Such filtrates contain the stimulatory interaction products. The bacteria were then transferred to newly collected filtrates by passing the media (or filtrates) containing the bacteria through a membrane filter so as to catch the bacteria on the surface of the filter. The filtrates from this were discarded. The filter was reversed and newly collected filtrates of media that contained the stimulatory interaction products were passed through to wash off the bacteria and suspend them in the filtrates.

No lag periods in the growth of the bacteria were observed in the transfer from filtrates containing interaction products to newly collected filtrates containing interaction products (Fig. 7-A, B). The absence of lag periods in this experiment is significant. This indicates

that the lag periods which were initially exhibited in filtrates containing interaction products are due to enzyme induction and not inhibition.

Bacteria were also incubated for 6 hrs in fresh media. They were then transferred to filtrates of media that previously contained mixtures of bacteria (10^6/ml) and enzyme-separated cells (5,000 µg/ml) for 12 hrs. Such filtrates contain stimulatory interaction products. Lag periods in growth did occur in such transfers. The lag periods were different in duration, depending on the source of enzyme-separated cells. The lag period was 2 hrs if the enzyme-separated cells were from the susceptible cv., Blackhawk (Fig. 7-A) and 4 to 6 hrs if the enzyme-separated cells were from the resistant cv., Lee (Fig. 7-B). This difference in lag periods indicates that the stimulatory substance(s) released or produced by the enzyme-separated cells of the susceptible and resistant cultivars are different in constituency or concentration. It cannot be concluded that their difference is due to the difference in susceptibility of the cultivars.

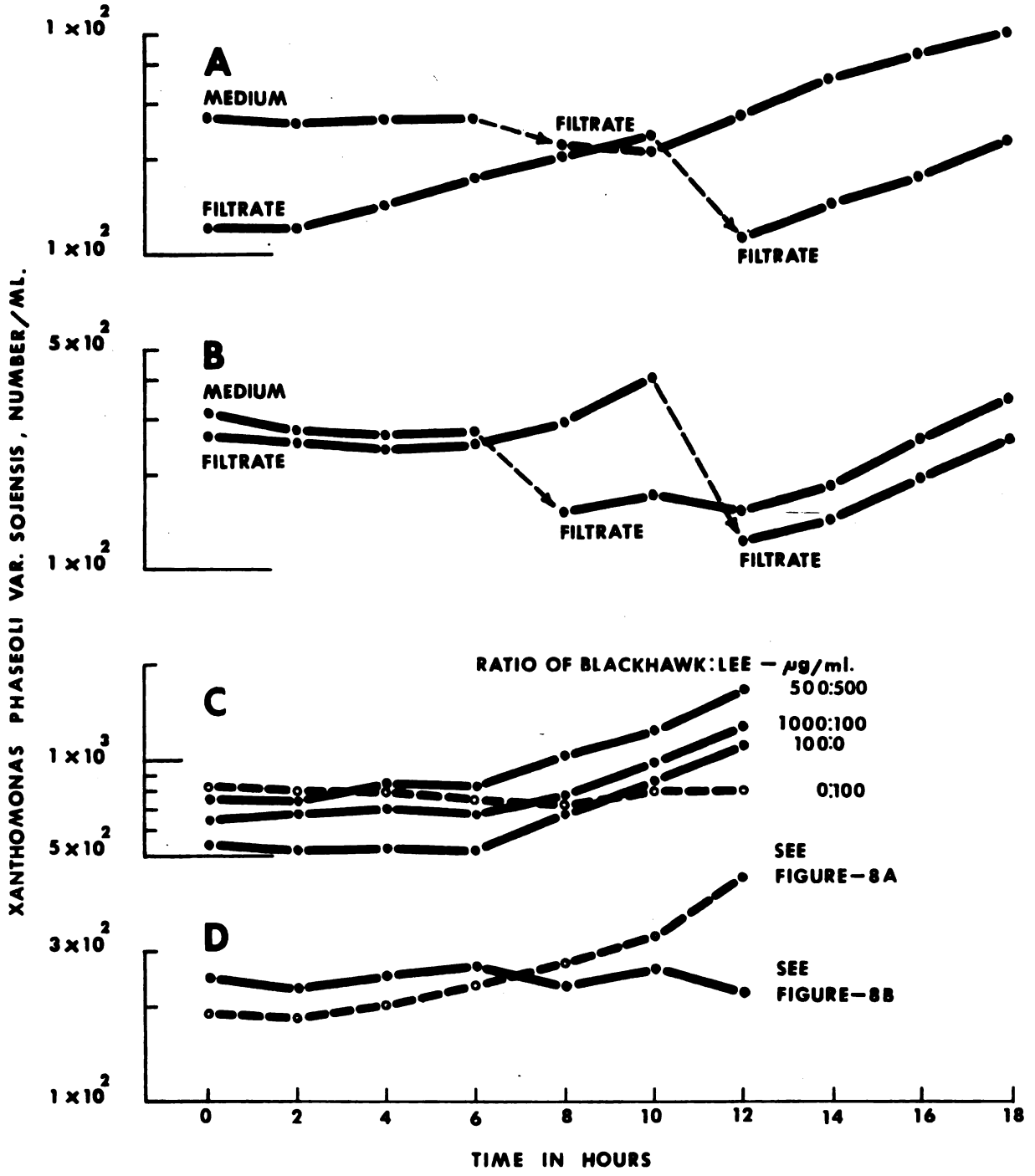
Awareness of the duration of the lag periods permits us to define the time of interaction more precisely. Growth of the parasite beings at the sixth hour of incubation with enzyme-separated cells of the susceptible cv. (Fig. 3-A). Because the duration of the lag period was 2 hrs the interaction must have occurred by the fourth hr.

FIGURE 7

(A - B) Studies on the lag phase of growth of Xanthomonas phaseoli var. sojensis in filtrates collected from media in which X. phaseoli var. sojensis (10^6 bacteria/ml) was incubated for 12 hrs with enzyme-separated cells (5,000 µg/ml) of (A) the susceptible cv. of soybean (Blackhawk) and (B) the resistant cv. (Lee). The dotted lines with arrows indicate when bacteria were transferred from medium to filtrate or filtrate to filtrate.

(C) Growth of X. phaseoli var. sojensis when incubated with mixtures of different concentrations of enzyme-separated cells of the susceptible (Blackhawk) and resistant (L33) cvs. of soybean.

(D) Growth of X. phaseoli var. sojensis in filtrates collected as diagrammed in Figure 8.


A
MEDIUM
FILTRATE
FILTRATE
FILTRATE
1 x 10²
1 x 10²
B
MEDIUM
FILTRATE
FILTRATE
FILTRATE
5 x 10²
1 x 10²
C
RATIO OF BLACKHAWK:LEE – µg/ml.
500:500
1000:100
100:0
0:100
1 x 10³
5 x 10²
SEE FIGURE–8A
D
SEE FIGURE–8B
3 x 10²
1 x 10²
0
2
4
6
8
10
12
14
16
18
TIME IN HOURS
XANTHOMONAS PHASEOLI VAR. SOJENSIS, NUMBER/ML.

On the other hand, growth of the parasite began at the eighth hr of incubation with host cells of the resistant cv. (Fig. 4-A). This interaction must have occurred by the second to fourth hour because the duration of the lag period was four to six hrs.

To determine whether or not inhibitory products are being produced by enzyme-separated cells of the resistant soybean cv., the following experiments were conducted. Growth rates of the parasite (X. phaseoli var. sojensis) were measured in media that contained mixtures of enzyme-separated cells of the resistant and susceptible soybean cv.

The growth rates of the parasite when incubated with cells of the susceptible soybean cv. were not affected by the presence of cells of the resistant soybean cv. (Fig. 7-C). Thus, an inhibitor does not appear to be present.

Previously, it was concluded that the parasite caused the enzyme-separated cells to release or produce substance(s) which stimulated the growth of the parasite. Further experiments to test this conclusion were conducted by growing the parasite in filtrates obtained with the procedures diagrammed in Figure 8.

If the parasite causes the host cells to release or produce the stimulatory substance(s), then culture filtrates of the bacteria might be expected to cause the same effect. Therefore, enzyme-separated cells were

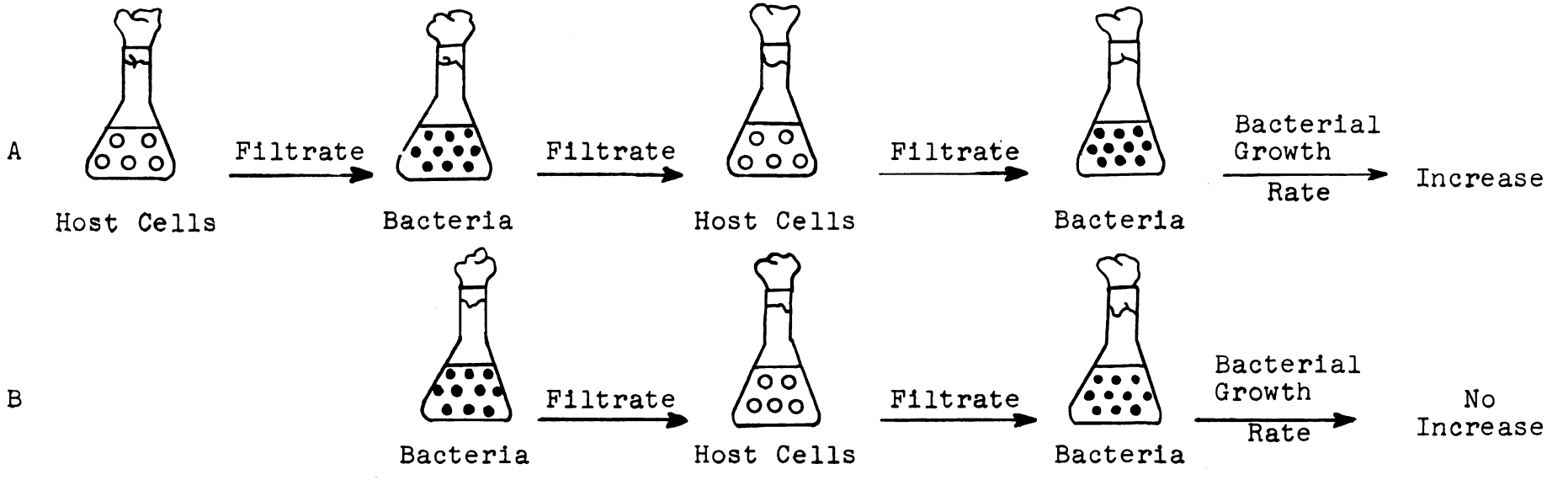
incubated for 12 hrs with filtrates of media which previously contained bacteria for 12 hrs. The host cells were removed by filtration and bacteria were added and incubated for 12 hrs (Fig. 8-B). No growth occurred (Fig. 7-D). It would appear that the host cells do not release or produce substance(s) under these conditions. It would not appear to be breakdown of the stimulatory substance(s) because the time periods of incubation of the host cells and the duration of their use was no longer than in the experiments in which the bacteria grew in filtrates of media that previously contained mixtures of bacteria and host cells.

An experiment was then conducted to determine if the bacterial products which cause the host cells to release stimulatory substance(s) are induced by the host cells. Therefore, enzyme-separated cells were incubated for 12 hrs in filtrates of media which contained the following; the host cells alone for 12 hrs, followed by the bacteria alone for 12 hrs. The host cells were removed by filtration and bacteria were added and incubated for 12 hrs (Fig. 8-A). The parasite grew (Fig. 7-D). Thus it appears that the enzyme-separated cells of the susceptible host induce the parasite to produce product(s) which cause the host cells to release substance(s) which stimulate the parasite to grow.

Xanthomonas phaseoli var. sojensis did not grow when incubated with enzyme-separated cells of N. tabacum,

FIGURE 8

Alternate and successive 12-hour incubation of Xanthomonas phaseoli var. sojensis (10^6 bacteria/ml) and enzyme-separated cells (5,000 µg/ml) of the susceptible cv. of soybean (Blackhawk). (A) Host cells incubated first, then bacteria, and then host cells, and (B) Bacteria incubated first and then host cells. The growth rate of X. phaseoli var. sojensis was measured in the last step.


A
Host Cells
Filtrate
Bacteria
Filtrate
Host Cells
Filtrate
Bacteria
Bacterial Growth Rate
Increase
B
Bacteria
Filtrate
Host Cells
Filtrate
Bacteria
Bacterial Growth Rate
No Increase

a nonhost (Fig. 9-B). Growth of Pseudomonas angulata increased when incubated with enzyme-separated cells of two hosts, soybean, cv. Lee and N. tabacum (Fig. 9-A).

Growth Rates of Bacterial Saprophytes When Incubated with Enzyme-Separated Cells of Soybean (cvs. Blackhawk and Lee)

To determine whether or not the host-parasite interactions observed with X. phaseoli var. sojensis and enzyme-separated cells of soybean (cvs. Blackhawk and Lee) were specific responses to a parasite and not general responses to any bacteria, the following study was conducted.

A saprophyte, S. marcescens, in concentrations from 10^1 to 10^6 bacteria/ml, was incubated with enzyme-separated cells of soybean (cvs. Blackhawk and Lee), at concentrations of 100 µg/ml.

Growth of the saprophyte occurred in the media alone after 8 hours of incubation (Figs. 10, B-C and 11, B-C). Similar patterns of growth were observed when 10^1 bacteria/ml were incubated with enzyme-separated cells of either cv. of soybean (Figs. 11-C; 12-C). Patterns of growth, however, were different when 10^2 bacteria/ml or higher concentrations were incubated with these plant cells (Figs. 10, A-B-C; 11, A-B-C). The bacteria began to grow sooner and grew at a faster rate. The fact that the rates of growth increased and occurred

FIGURE 9

(A) Growth of Pseudomonas angulata in the incubation medium alone and with enzyme-separated cells (100 µg/ml) of susceptible hosts--soybean, cv. Lee and Nicotiana tabacum.

(B) Growth of Xanthomonas phaseoli var. sojensis when incubated with enzyme-separated cells (100 µg/ml) of a non-host, N. tabacum.

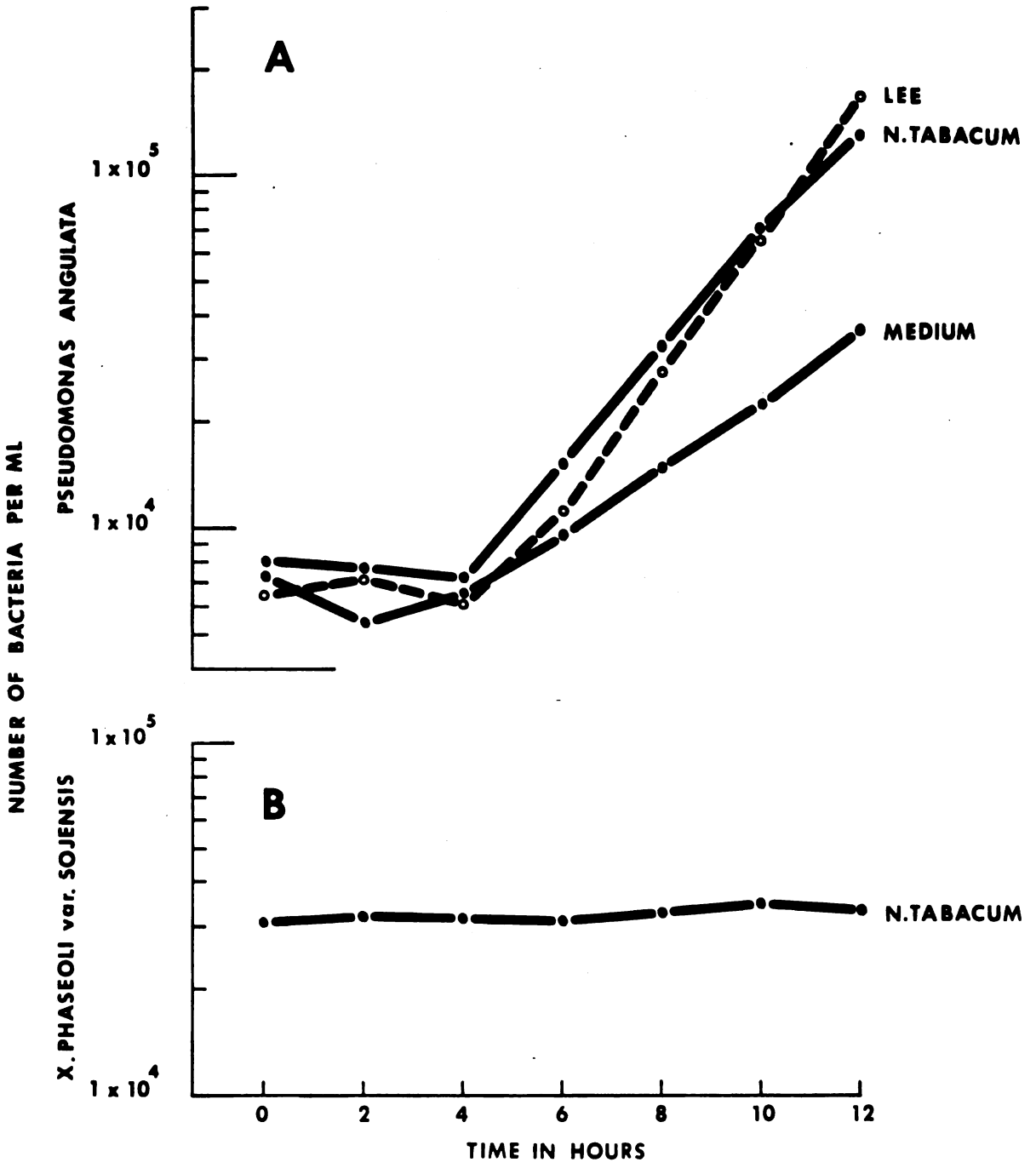

A
LEE
N.TABACUM
MEDIUM
1 x 10^5
1 x 10^4
PSEUDOMONAS ANGULATA
B
N.TABACUM
1 x 10^5
1 x 10^4
X. PHASEOLI var. SOJENSIS
NUMBER OF BACTERIA PER ML
0
2
4
6
8
10
12
TIME IN HOURS

FIGURE 10

Growth of Serratia marcescens when incubated in the medium alone and with enzyme-separated cells (100 µg/ml) of soybean cv. Blackhawk. Various concentrations of bacteria were used.

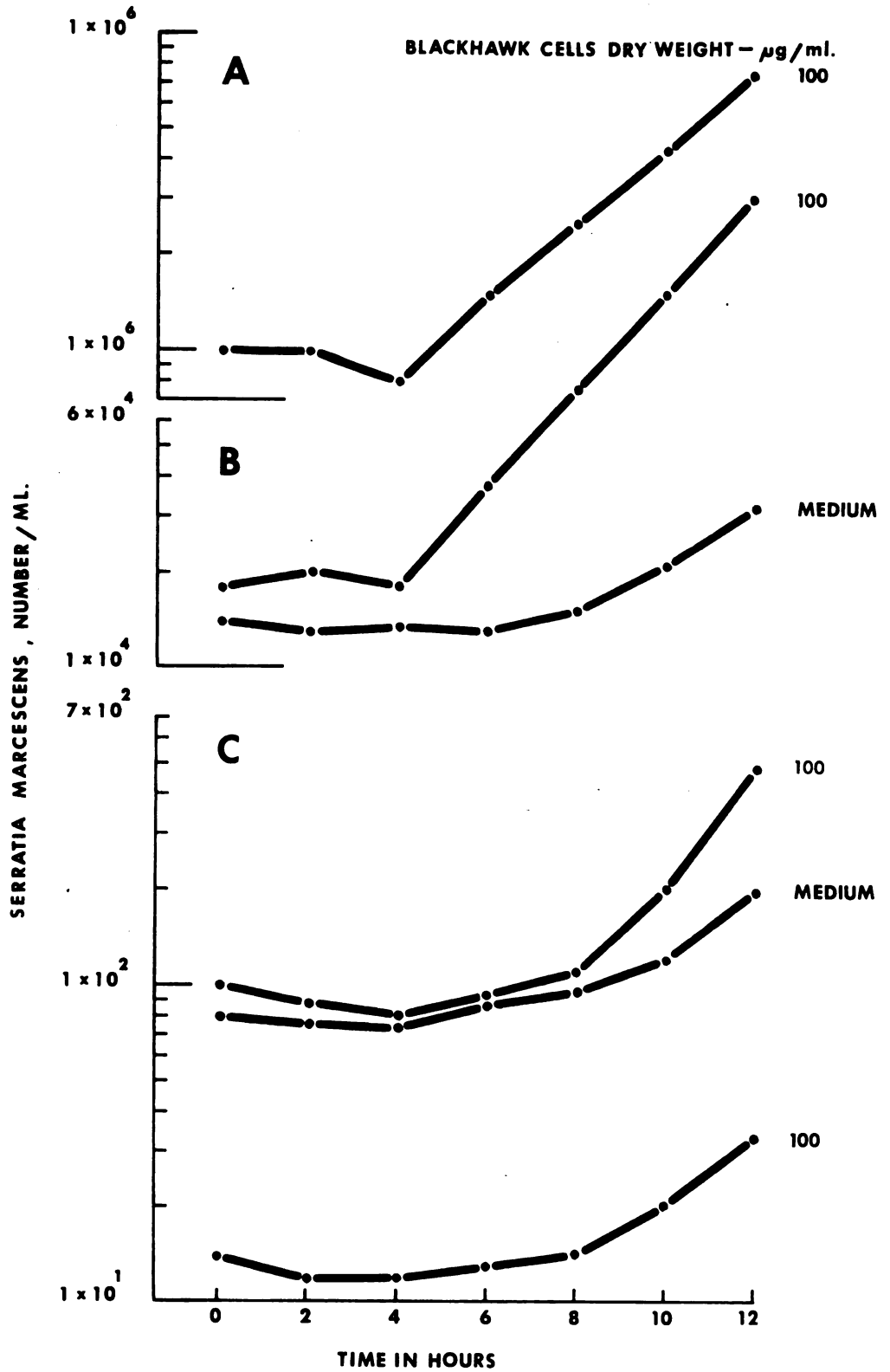
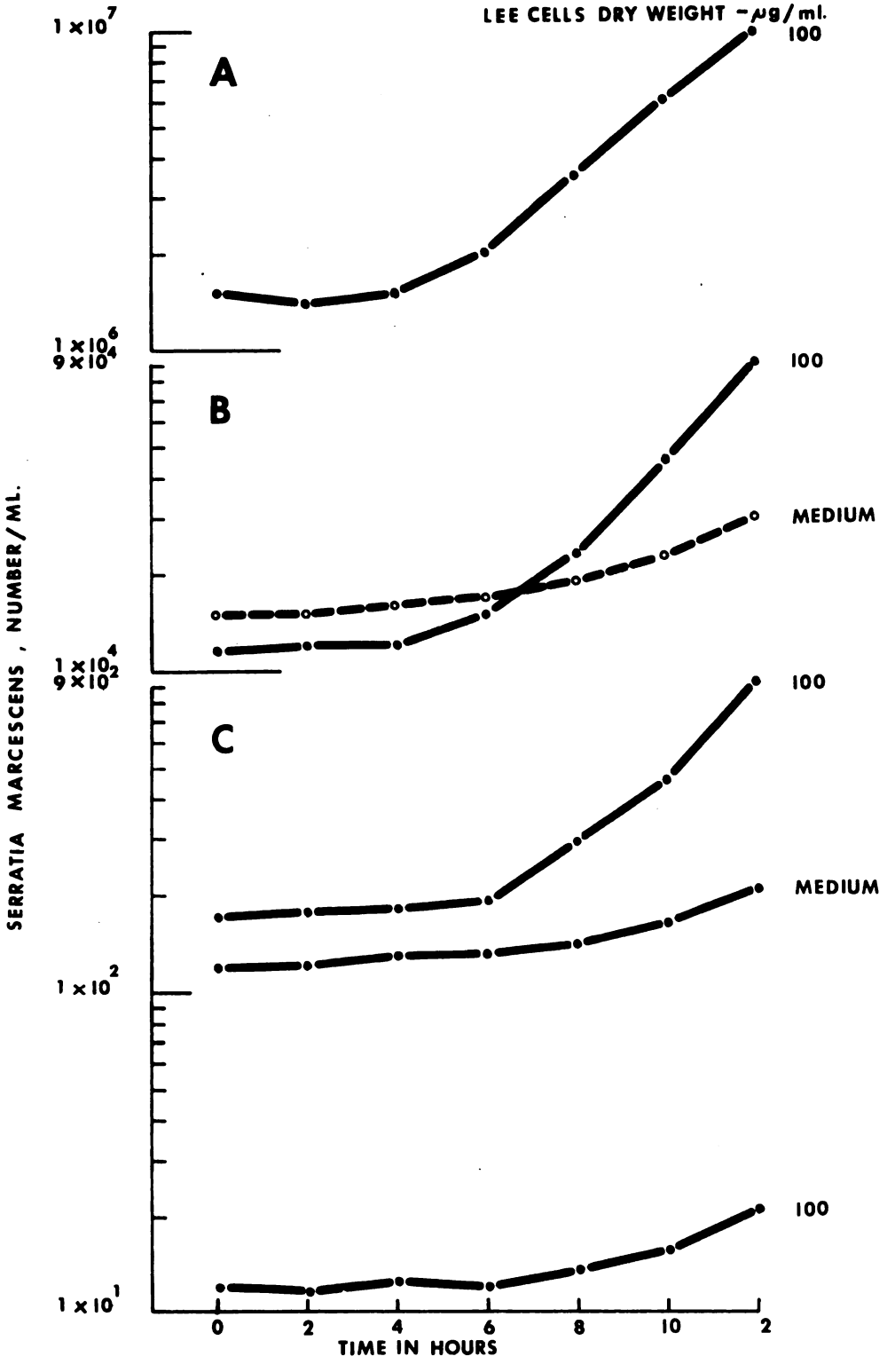

BLACKHAWK CELLS DRY WEIGHT – µg/ml.
A
B
C
100
100
MEDIUM
100
MEDIUM
100
1 x 10^6
1 x 10^6
6 x 10^4
1 x 10^4
7 x 10^2
1 x 10^2
1 x 10^1
SERRATIA MARCESCENS, NUMBER/ML.
0
2
4
6
8
10
12
TIME IN HOURS

FIGURE 11

Growth of Serratia marcescens when incubated in the medium alone and with enzyme-separated cells (100 µg/ml) of soybean cv. Lee. Various concentrations of bacteria were used.


LEE CELLS DRY WEIGHT - µg/ml.
1 x 10^7
A
100
1 x 10^6
9 x 10^4
B
100
MEDIUM
1 x 10^4
9 x 10^2
C
100
MEDIUM
1 x 10^2
100
1 x 10^1
SERRATIA MARCESCENS , NUMBER/ML.
0
2
4
6
8
10
2
TIME IN HOURS

earlier indicates that the plant cells released or produced stimulatory substances in response to the saprophyte.

Additional experiments were performed to determine if the stimulatory substance(s) were products of interactions. A saprophyte, S. marcescens, in concentrations of 10^2 and 10^4 bacteria/ml, was incubated with enzyme-separated cells of soybean (cvs. Blackhawk and Lee) in concentrations from 5 to 5,000 µg/ml. The bacteria began to grow sooner and at a faster rate as the concentrations of both the saprophytic bacteria and enzyme-separated cells of either cv. of soybean were increased (Figs. 12, A-B; 13, A-B).

The earlier and increased rate of growth of the saprophyte when incubated with enzyme-separated cells (than when incubated in media alone), indicate that stimulatory substance(s) are released or produced by the soybean cells. It would seem that the saprophyte causes the host cells to release or produce the stimulatory substance(s) because the number of saprophytic bacteria and enzyme-separated cells affect the initiation and rate of growth of the bacteria. These stimulatory substance(s) then are products of interaction of the saprophyte and plant cells. They may or may not be different from those which these plant cells released or produced when incubated with the parasite, X. phaseoli var. sojensis, as described earlier.

FIGURE 12

Growth of Serratia marcescens when incubated with enzyme-separated cells of soybean cv. Blackhawk. Various concentrations of bacteria and plant cells were used.

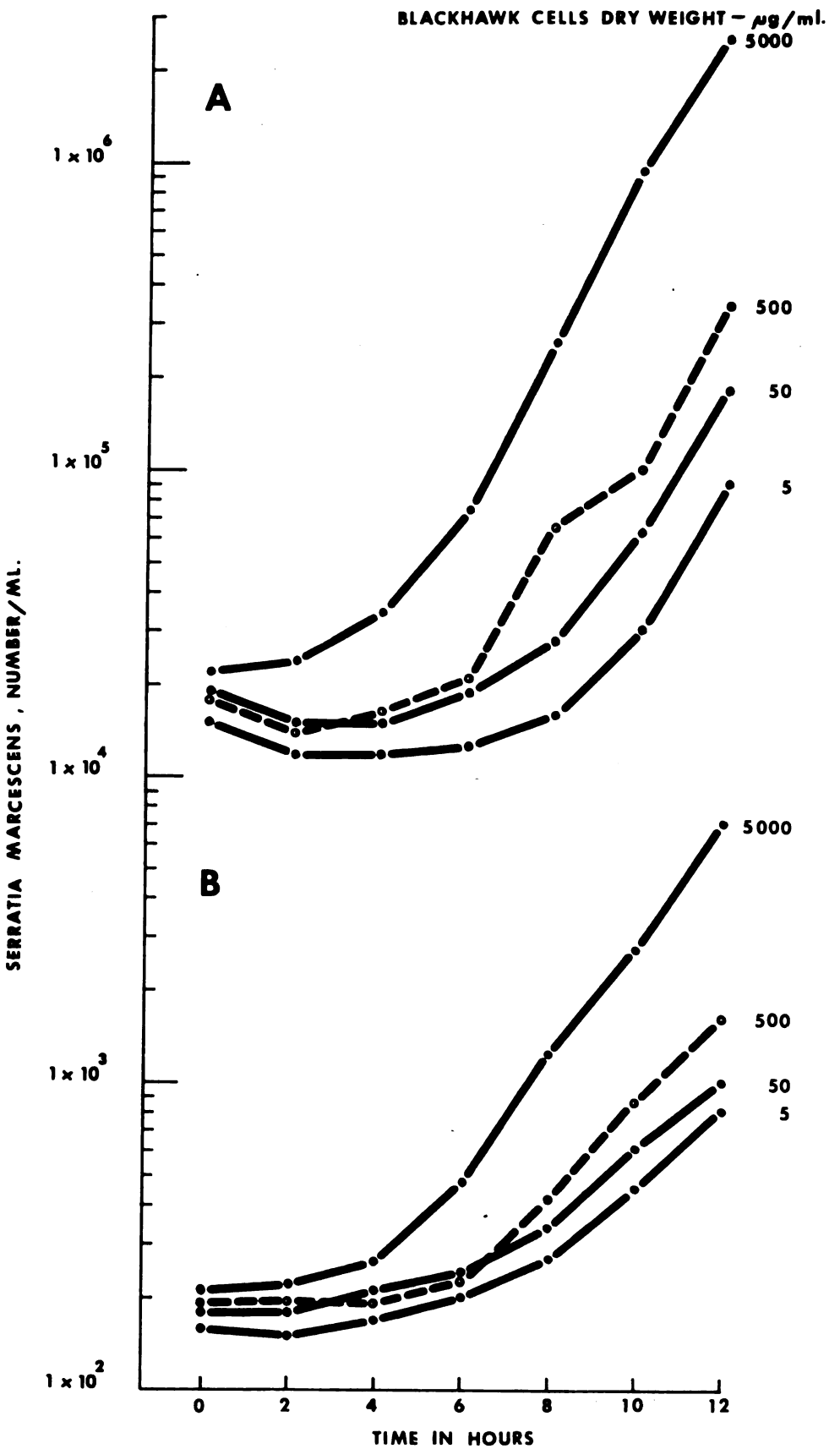
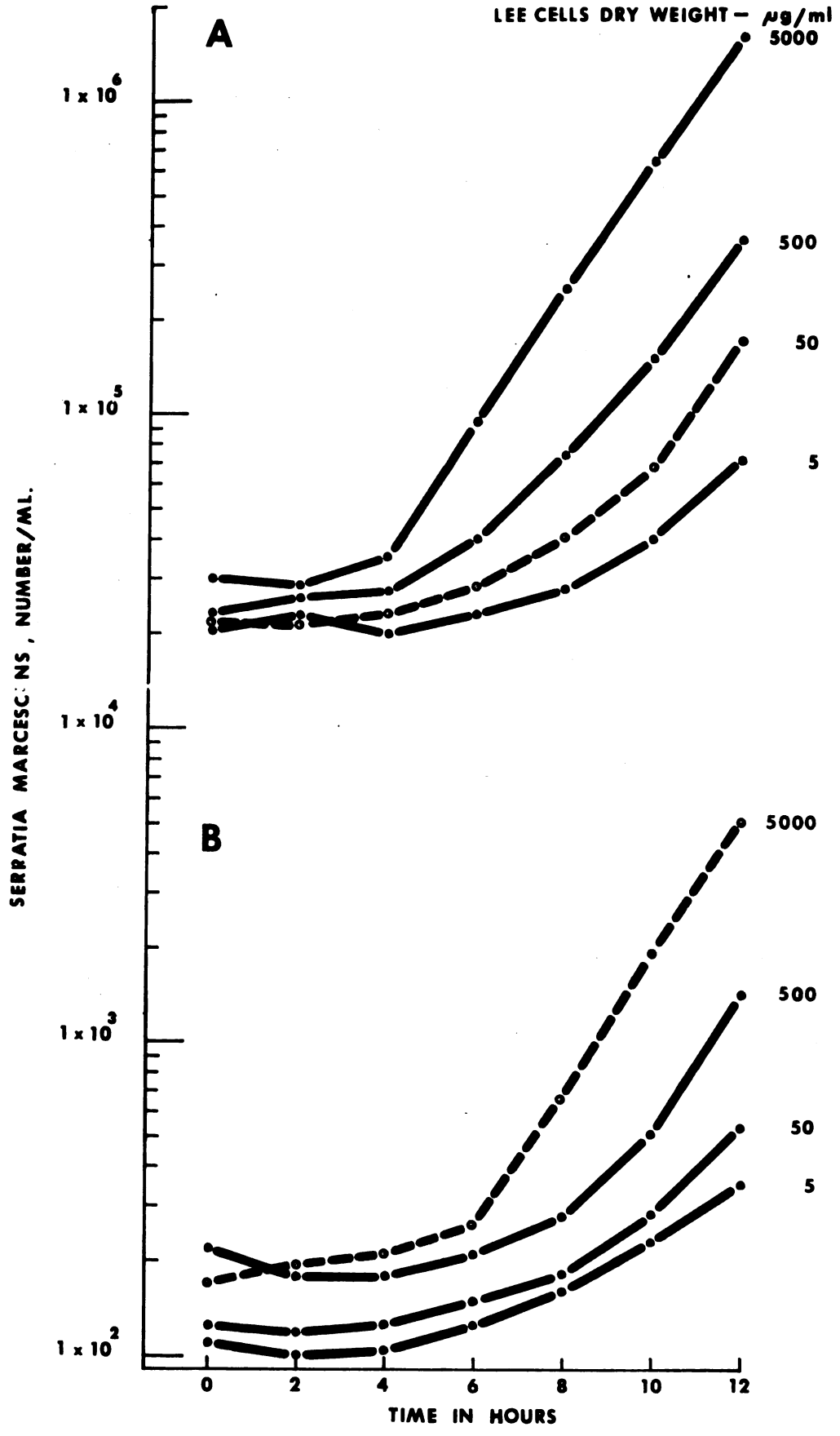

BLACKHAWK CELLS DRY WEIGHT – µg/ml.
A
B
5000
500
50
5
1 x 10^6
1 x 10^5
1 x 10^4
1 x 10^3
1 x 10^2
SERRATIA MARCESCENS, NUMBER/ML.
0
2
4
6
8
10
12
TIME IN HOURS

FIGURE 13

Growth of Serratia marcescens when incubated with enzyme-separated cells of soybean cv. Lee. Various concentrations of bacteria and plant cells were used.


LEE CELLS DRY WEIGHT — µg/ml
A
5000
500
50
5
B
5000
500
50
5
1 x 10^6
1 x 10^5
1 x 10^4
1 x 10^3
1 x 10^2
SERRATIA MARCESCENS, NUMBER/ML.
0
2
4
6
8
10
12
TIME IN HOURS

Growth Rates of S. marcescens in Filtrates

To determine whether or not the stimulatory substances were products of interaction between the saprophyte and soybean cells, the following experiments were conducted. The saprophyte (S. marcescens) was grown in filtrates from media which previously contained either: (1) only the saprophyte (10^4 bacteria/ml), (2) only enzyme-separated cells (5,000 µg/ml), or (3) mixtures of the two. Filtrates were collected and sterilized by filtration after every two hrs beginning at 0.2 hrs and ending at 12 hrs.

The saprophyte (S. marcescens) grew in the filtrates of media which previously contained the saprophyte. This growth was no different than that of the saprophyte in the media alone (Fig. 14). Growth was increased, however, in filtrates of media which previously contained enzyme-separated cells of both the Blackhawk and Lee cvs. of soybean for 0.2, 2 or 12 hrs (Figs. 15-B, 16-B). This clearly indicates that the plant cells of both cvs. of soybean leaked substance(s) in 0.2 hrs which stimulated the growth of the saprophyte. The parasite, X. phaseoli var. sojensis did not grow in such filtrates (Figs. 3-B, 4-B). This demonstrates that X. phaseoli var. sojensis and S. marcescens have different growth requirements. No conclusions are warranted which relate these differences to host-parasite interactions.

FIGURE 14

Growth of Serratia marcescens (A, B) in medium alone, and (C) in filtrates from media in which S. marcescens (10^4 bacteria/ml) was incubated for 0.2 and 12 hrs.

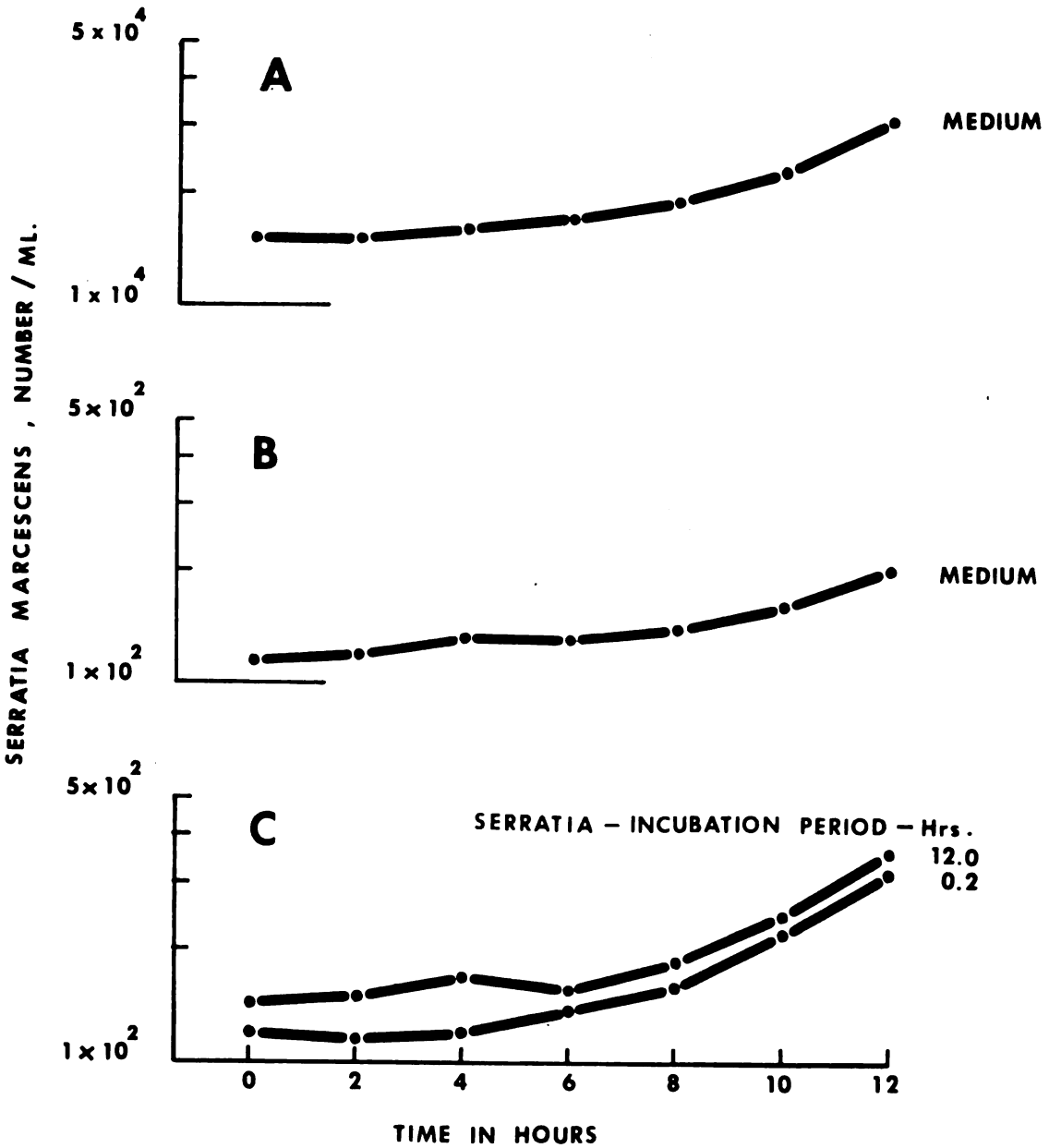

5 x 10^4
A
MEDIUM
1 x 10^4
5 x 10^2
B
MEDIUM
1 x 10^2
5 x 10^2
C
SERRATIA – INCUBATION PERIOD – Hrs.
12.0
0.2
1 x 10^2
0
2
4
6
8
10
12
TIME IN HOURS
SERRATIA MARCESCENS , NUMBER / ML.

FIGURE 15

Growth of Serratia marcescens in filtrates of media in which were incubated for 0.2 to 12 hrs, (A) a mixture of enzyme-separated cells (5,000 µg/ml) of soybean cv. Blackhawk, and S. marcescens (10^4 bacteria/ml); and, (B) only enzyme-separated cells (5,000 µg/ml) of soybean cv. Blackhawk.

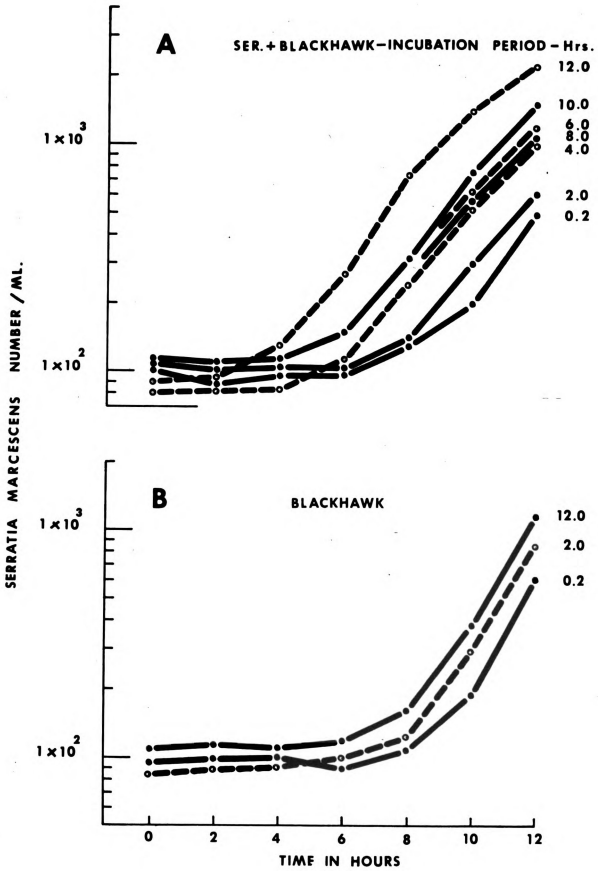
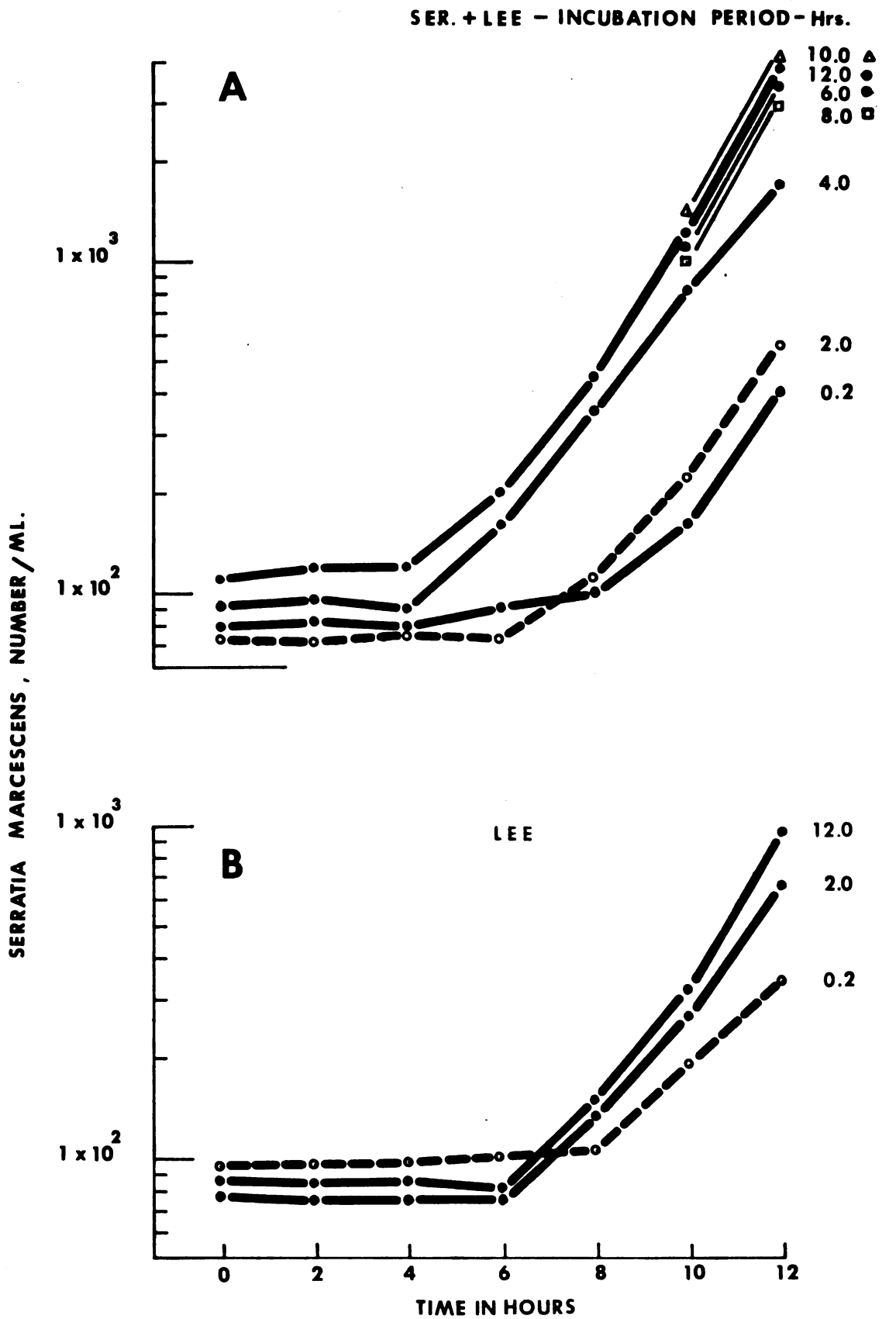

A
SER. + BLACKHAWK – INCUBATION PERIOD – Hrs.
12.0
10.0
6.0
8.0
4.0
2.0
0.2
1×10³
1×10²
B
BLACKHAWK
12.0
2.0
0.2
1×10³
1×10²
SERRATIA MARCESCENS NUMBER / ML.
0
2
4
6
8
10
12
TIME IN HOURS

FIGURE 16

Growth of Serratia marcescens in filtrates of media in which were incubated for 0.2 to 12 hrs, (A) a mixture of enzyme-separated cells (5,000 µg/ml) of soybean cv. Lee and S. marcescens (10^4 bacteria/ml); and, (B) only enzyme-separated cells (5,000 µg/ml) of soybean cv. Lee.


SER. + LEE – INCUBATION PERIOD – Hrs.
A
10.0
12.0
6.0
8.0
4.0
2.0
0.2
1 x 10^3
1 x 10^2
SERRATIA MARCESCENS, NUMBER/ML.
B
LEE
12.0
2.0
0.2
1 x 10^3
1 x 10^2
0
2
4
6
8
10
12
TIME IN HOURS

Growth of the saprophyte (S. marcescens) in filtrates of media which previously contained mixtures of the saprophyte and enzyme-separated cells for 0.2 to 2 hrs (Figs. 15-A, 16-A) was no different than in filtrates of media which previously contained only enzyme-separated cells (Figs. 15-B, 16-B). Growth commenced after a lag period of 6 to 8 hrs. Growth, however, in filtrates of media which previously contained the mixtures for longer than 2 hrs, commenced sooner--after a lag period of 2 to 4 hrs. This earlier commencement of growth was due to stimulatory products of interaction between the saprophyte and plant cells. The interaction occurred before the fourth hr. It is reasonable to conclude that the saprophyte caused the plant cells to release or produce these stimulatory substance(s).

To determine the duration of the lag period in growth of the saprophyte in filtrates that contain interaction product(s), the following experiments were conducted. Bacteria were incubated for 8 hrs in filtrates of media that previously contained mixtures of bacteria (10^4/ml) and enzyme-separated cells (5,000 µg/ml) for 12 hrs. Such filtrates contain interaction products. The bacteria were transferred to newly collected filtrates and to fresh media as previously described in similar experiments with the parasite.

No lag periods in the growth of the bacteria were observed in the transfer of bacteria from filtrates

containing interaction products to newly collected filtrates containing interaction products (Fig. 17-A). The absence of the lag periods in this experiment is significant. This indicates that the lag periods in growth of the bacteria are due to enzyme induction and not inhibition.

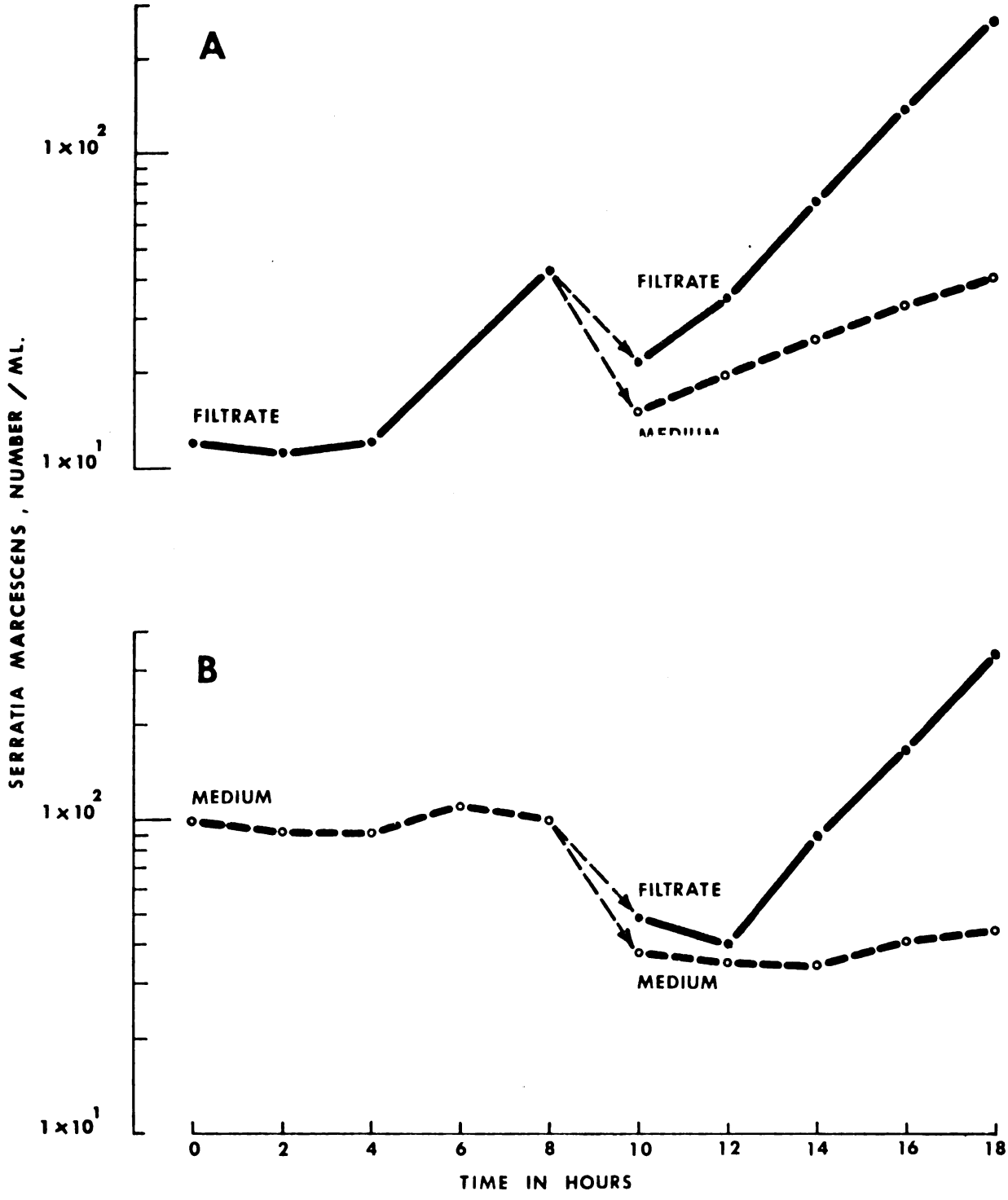
Bacteria were also incubated for 6 hrs in fresh media. They were then transferred to fresh media and filtrates of media that previously contained mixtures of bacteria (10^4 /ml) and enzyme-separated cells (5,000 µg/ml) for 12 hrs. Such filtrates contain interaction products. Lag periods of growth did occur when the bacteria were transferred to filtrates that contain the interaction products (Fig. 17-B). The lag period was 2 hrs.

Awareness of the duration of the lag period permits us to define the time of interactions more clearly. Growth of the saprophyte began between the second and fourth hr of incubation with enzyme-separated cells of soybean (Figs. 12-A, 13-A). The stimulatory substance(s) which caused the growth must have been present prior to the lag period of 2 hrs. The interaction that brought about the stimulatory substance(s) must have occurred between 0 and 2 hours of incubation.

Previously, it was concluded that the saprophyte caused the enzyme-separated cells to release or produce substances which stimulated the growth of the saprophyte. Further experiments to test this conclusion were

FIGURE 17

Studies on the lag phase of growth of Serratia marcescens in filtrates collected from media in which S. marcescens (10^4 bacteria/ml) was incubated for 12 hrs with enzyme-separated cells (5,000 µg/ml) of soybean, cv. Lee. Dotted lines with arrows indicate when the bacteria were transferred from (A) filtrates to filtrates and media, and (B) media to filtrates and media.


A
B
FILTRATE
FILTRATE
MEDIUM
MEDIUM
FILTRATE
MEDIUM
1 x 10^2
1 x 10^1
1 x 10^2
1 x 10^1
SERRATIA MARCESCENS, NUMBER / ML.
0
2
4
6
8
10
12
14
16
18
TIME IN HOURS

conducted by growing the saprophyte in filtrates obtained with the procedures diagrammed in Figure 8.

If the saprophyte causes the enzyme-separated cells of soybean to release or produce the stimulatory substance(s), the culture filtrates of the bacteria might be expected to cause the same effect. Therefore, enzyme-separated cells were incubated for 12 hrs with filtrates of media which previously contained bacteria for 12 hrs. The enzyme-separated cells were removed by filtration and bacteria were added and incubated for 12 hrs (Fig. 18). The saprophyte grew in this filtrate after four hrs of incubation (Fig. 19-A). Thus it appears that the soybean cells release or produce stimulatory substance(s) under these conditions. The saprophyte grew in this filtrate at the same rate as it did in a filtrate of media which previously contained mixtures of the bacteria and the enzyme-separated cells (Fig. 19-B).

To determine whether or not the interaction products which stimulated the saprophyte (S. marcescens) to grow were the same as the products which stimulated the parasite (X. phaseoli var. sojensis) to grow, the following experiment was conducted. Growth rates of the parasite and saprophyte were measured in two filtrates; (1) filtrates from media which previously contained mixtures of enzyme-separated cells (5,000 µg/ml) of soybean (cv. Lee) and the saprophyte (10^6 bacteria/ml) for 12 hrs, and (2) filtrates of media which previously contained mixtures

FIGURE 18

Alternate and successive 12-hr incubation of Serratia marcescens (10^4 bacteria/ml) and enzyme-separated cells (5,000 µg/ml) of soybean, cv. Lee. Growth of S. marcescens was measured in the last step.

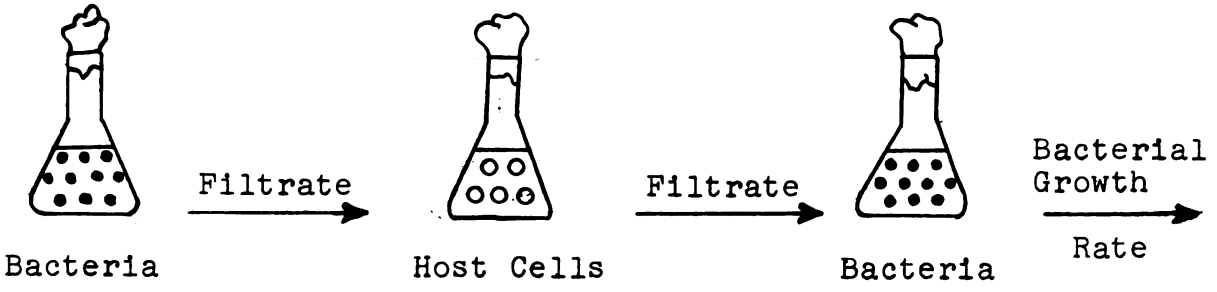
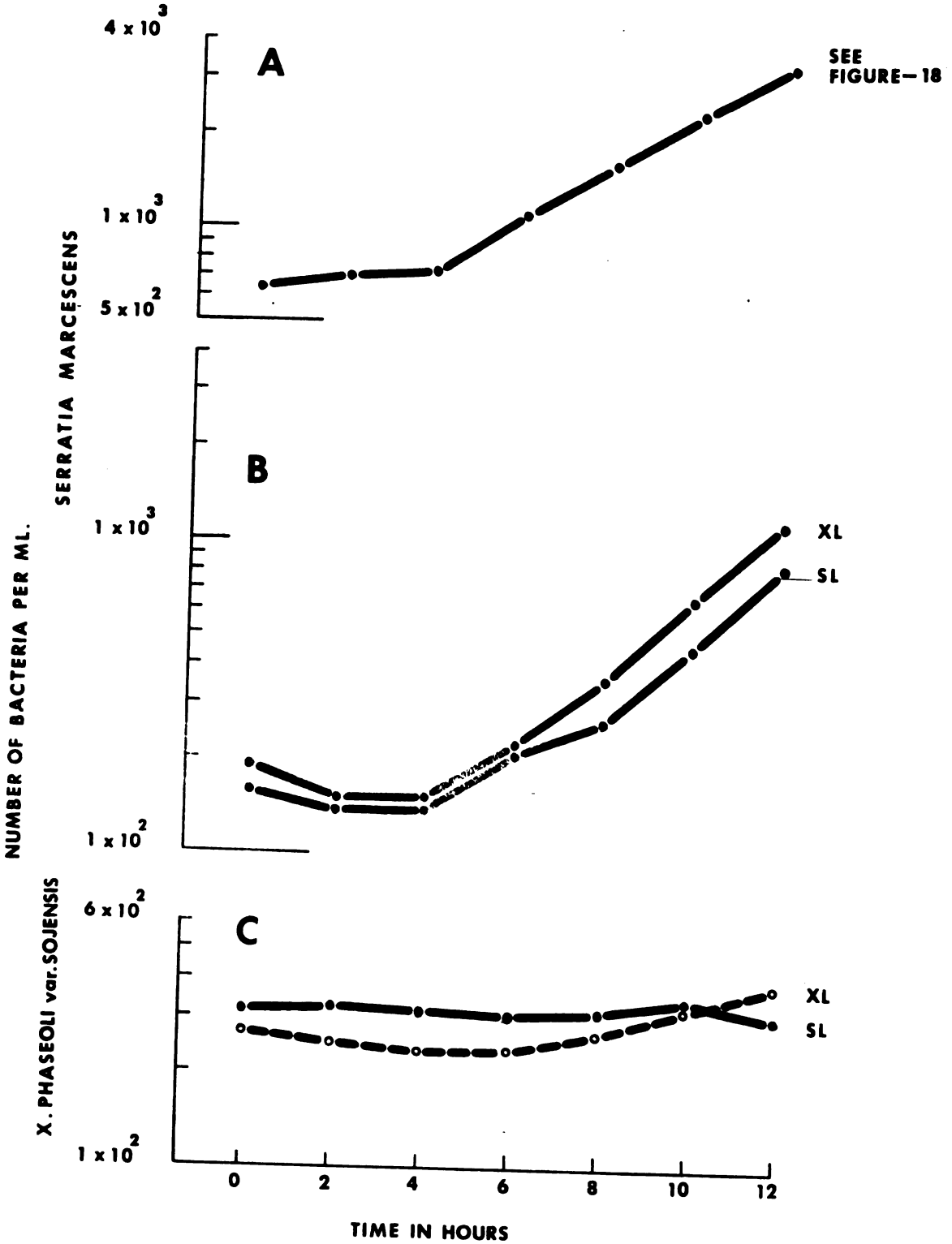

Filtrate
Filtrate
Bacterial
Growth
Rate
Increase
Bacteria
Host Cells
Bacteria

FIGURE 19

(A, B) Growth of Serratia marcescens in filtrates collected (A) as diagrammed in Figure 18, and (B) from media in which enzyme-separated cells (5,000 µg/ml) of soybean cv. Lee were incubated for 12 hrs with S. marcescens (10^4 bacteria/ml) (SL) and Xanthomonas phaseoli var. sojensis (10^6 bacteria/ml) (XL). (C) Growth of X. phaseoli var. sojensis in filtrates from media in which enzyme-separated cells (5,000 µg/ml) of soybean cv. Lee were incubated for 12 hrs with S. marcescens (10^6 bacteria/ml) (SL) and X. phaseoli var. sojensis (10^6 bacteria/ml) (XL).


A
B
C
SEE FIGURE—18
SERRATIA MARCESCENS
X. PHASEOLI var. SOJENSIS
NUMBER OF BACTERIA PER ML.
4 x 10^3
1 x 10^3
5 x 10^2
1 x 10^3
1 x 10^2
6 x 10^2
1 x 10^2
XL
SL
XL
SL
0
2
4
6
8
10
12
TIME IN HOURS

of the similar enzyme-separated cells and the parasite (10^6 bacteria/ml).

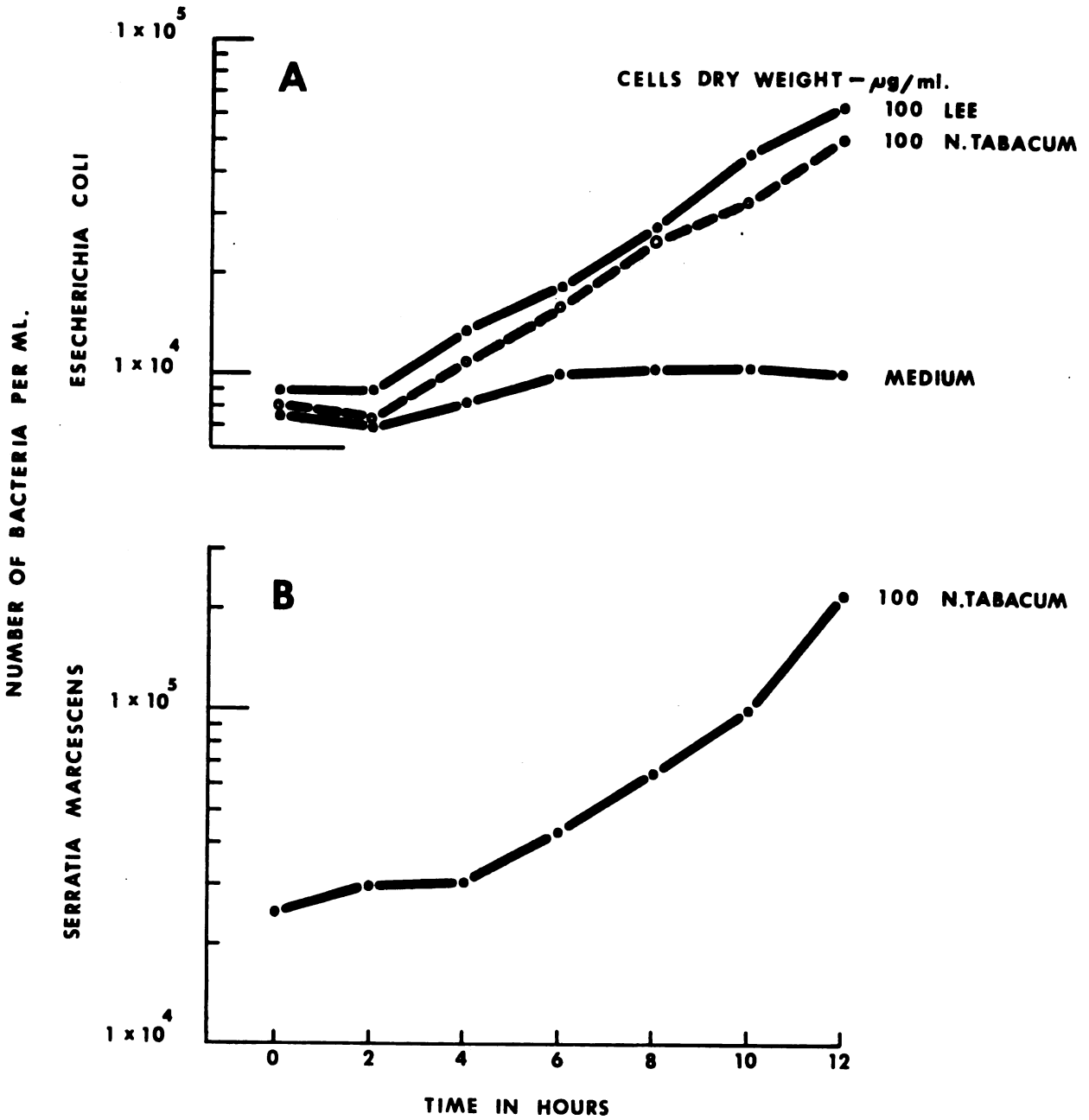
The parasite grew in the filtrates of media which previously contained mixtures of the host cells and the parasite but not in media which previously contained mixtures of similar plant cells and the saprophyte (Fig. 19-C). The saprophyte, on the other hand, grew equally well in both kinds of filtrates (Fig. 19-B). The results with the saprophyte indicate that both the saprophyte and the parasite cause the enzyme-separated cells of soybean, cv. Lee, to release or produce substance(s). The results with the parasite indicate either; (1) that these substance(s) are different, in part or in whole, (2) that the saprophyte produces antibiotics that inhibit the parasite, or (3) that the saprophyte has depleted the stimulatory substance(s).

The growth of the saprophyte (S. marcescens) also increased when the bacteria were incubated with enzyme-separated cells of leaves of tobacco (N. tabacum) (Fig. 20-B). The growth of another saprophyte, E. coli, also increased when the bacteria were incubated with enzyme-separated cells of soybean (cv. Lee) and N. tabacum (Fig. 20-A).

FIGURE 20

(A) Growth of Escherichia coli when incubated in media alone and with enzyme-separated cells (100 µg/ml) of soybean, cv. Lee and Nicotiana tabacum.

(B) Growth of Serratia marcescens when incubated with enzyme-separated cells (100 µg/ml) of N. tabacum.


NUMBER OF BACTERIA PER ML.
ESECHERICHIA COLI
1 x 10^5
1 x 10^4
A
CELLS DRY WEIGHT – µg/ml.
100 LEE
100 N.TABACUM
MEDIUM
SERRATIA MARCESCENS
1 x 10^5
1 x 10^4
B
100 N.TABACUM
0
2
4
6
8
10
12
TIME IN HOURS

DISCUSSION

Use of Enzyme-Separated Cells in Host-Parasite Studies

Advantages

The suspension of bacteria and enzyme-separated plant cells in a common medium provides for uniformity of interaction of all cells of both participants. Uniformity of interactions is possible because the cells of both participants are individual and separated. They have, therefore, equal opportunity to react to substances which each releases into the medium. This multiplies the products of interaction and synchronizes their occurrence. Multiplication of the products of interaction permit their detection at very short time intervals. This is evidenced by the detection of three interactions between X. phaseoli var. sojensis and the cells of the susceptible host in 4 hours.

Disadvantages

The number of viable cells decreases after 36 hrs (27). Therefore, experiments conducted after 36 hrs must be interpreted carefully.

The metabolism of enzyme-separated cells differs from intact tissue. The cells do not synthesize protein

(38), loose 80% of their photosynthetic capacity after 1 hr (38), contain only 25% of the proteins originally found in the intact plant (27), and respire at a lower rate (15). Although the respiration rate of enzyme-separated soybean cells is only 8% of a corresponding amount of intact tissue, differences in respiration of 160 µl/hrs/mg dry weight have been observed between enzyme-separated cells when incubated with and without the parasite. Filtrates of media which contained the host cells and the parasite for 12 hrs also gave similar results (15).

Interactions Occur

These studies indicate that interactions occur between bacteria and enzyme-separated cells of plant tissues. Whether or not these interactions occurred depended on the species and numbers of bacteria and plant cells. The nature of the interactions depended on the species of bacteria and plants. Such interactions can be compared to chemical reactions. The rates of both are determined by the concentrations of the reactants. The enzyme-separated cells and bacteria represent the reactants. The products of interactions represent the products of the chemical reactions and both depend on the nature of the reactants.

Simple chemical reactions, however, do not explain why these interactions occur only with a minimal, or

threshold number of bacteria and enzyme-separated cells. It could be that the metabolic products produced by the bacteria and enzyme-separated cells are breaking down and excesses are needed to make up for the losses. Excesses may also be needed to first fulfill the requirements of other metabolic pathways.

Interactions Are Different

Different interactions occurred between cells of a resistant (soybean cv. Lee) and cells of a susceptible host (soybean cv. Blackhawk) when incubated with a parasite (X. phaseoli var. sojensis). The interactions were different because 100 times as many bacteria and 100 times as many host cells were required to produce the interactions between cells of the resistant host and the parasite. The products of the interactions were also different in constituency or concentration because filtrates containing the interaction product(s) of the resistant host-parasite combination took a longer period of time to stimulate the parasite to grow. The parasite (X. phaseoli var. sojensis) did not grow when incubated with a non host (N. tabacum) while another parasite (Pseudomonas angulata) did grow when incubated with enzyme-separated cells of a susceptible host (soybean cv. Lee or N. tabacum).

Significance of Interactions

The results of experiments with S. marcescens and enzyme-separated plant cells indicate that interactions occur between them. The interactions appeared to be similar whether the host cells were the Lee cv. or whether they were the Blackhawk cv. of soybean. These interactions, however, were different from the interactions of X. phaseoli var. sojensis with these same cvs. Lower concentrations of the saprophyte and enzyme-separated cells were required to produce the interaction. The interaction between the saprophyte and the enzyme-separated soybean cells occurred 2 hrs sooner. The interaction products could be different because the parasite did not grow in filtrates that contained interaction products of the saprophyte and soybean cells. These differences, however, could also be explained by: (1) the presence of an inhibitor produced by the saprophyte, or (2) depletion of the stimulatory interaction product(s) by the saprophyte. Differences in the initiation of interactions were also observed. The saprophytic bacteria were able to make the plant cells release or produce substances stimulatory to their growth. In contrast, the parasitic bacteria could not make the host cells release or produce substances stimulatory to their growth unless first exposed to the filtrates of media that previously contained the host cells. Differences in bacterial growth were also

observed between other saprophyte-plant cell and parasite-host cell combinations (E. coli and P. angulata with tobacco).

Significance of Different Interactions

The differences in the interactions of a parasite when incubated with cells of a resistant and susceptible cv. appear to reflect differences in the cvs. The difference in the cvs. are not expressed in the interactions with the saprophyte. The interactions of both cvs. when incubated with the saprophyte, however, are different than either cv. with the parasite. Therefore the difference between the cvs. appears to reflect a host-parasite interaction.

Integration of this work with others is difficult. Other workers measured bacterial growth at 24-hr intervals (2, 9, 16, 29), whereas I measured bacterial growth every 2 hrs up to 12 hrs. There are no data on the growth of bacteria from 0 to 24 hours in intact tissues or living cells of plant tissues. The data herein, however, should be compared with data obtained using intact plant tissues and tissue culture systems. Single cell tissue culture systems would have the added advantage of determining the rate of multiplication of the host cells as well as that of the parasite. This system of viable and dividing cells would also permit studies beyond 12 hrs.

Once the number of interactions between the host and parasite are known and the significance of these interactions determined, the compounds involved in the interactions should be investigated. Since the minimal nutritive requirements for the genus Xanthomonas are simple and consist of a carbon source of glucose, salts and glutamic acid or methionine, these nutrient requirements could undoubtedly be met by the tissues of practically any plant (47). Thus according to Starr (47),

> Why, then, are these phytopathogenic bacteria restricted to a specific, or to a limited series of, host plants? The answer clearly does not lie in simple satisfaction of the minimal nutritive requirements of the pathogen.

Thus, the compounds which stimulate the bacteria to grow should be defined but their role in the success or failure of the parasite is open to question. The compound(s) which initiate the initial interaction, along with those that follow, possibly hold the key to parasitism and should be thoroughly investigated. Hopefully the complete series of chemical reactions which determine the success or failure of the parasite can be analyzed.

If the interactions of cells of resistant and susceptible plants with parasites observed in this study are due to the resistance and susceptibility of the plant cells, then this enzyme-separated cell system can also be used as a model to study other host-parasite interactions.

This system may have other applications as well. Membrane permeability could be studied because the enzyme-separated cells leak substance(s) as do the cells of intact pieces of plant tissues (8). The multiplication of saprophytes in plant tissues could be studied. A knowledge of the ecology of these organisms could be important in controlling foliar diseases of plants (17, 32, 33).

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