7/84 to 6/86

1986/DAQ11 44

COTTON RESEARCH COUNCIL

(FINAL REPORT)

Project Number: DAQ11

Project Title: The role of plant stress in the development of bacterial blight (Xanthomonas campestris pv. malvacearum) in cotton.

Field of Research: Plant Disease Field Code: 5

Organisation: Queensland Department of Primary Industries

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Commencement Date: July 1984 Completion Date: June 1986

Objectives: To assess whether plant stress as related to a nutritional imbalance caused by too high or too low a concentration of nitrogen, phosphorus or potassium influenced the severity of bacterial blight.

INTRODUCTION

Bacterial blight caused by the bacterium Xanthomonas campestris pv. malvacearum (Smith 1901) Dye 1978 is a major disease of cotton in Australia. Since this disease was first recorded early in the 20th century, a considerable amount of research has been carried out to determine the factors which influence the development of the disease. Despite the fact that specific environmental conditions favouring infection and disease development are known, the unpredictable and sudden severe outbreaks of the disease at isolated sites within plantings in Queensland crops had not been satisfactorily explained. It is possible that nutritional or water stress may be contributing factors in predisposing plants to a rapid build up of the pathogen within the plant thus increasing the severity of bacterial blight.

The experiments reported here investigated the influence of high or low concentrations of nitrogen, phosphorus and potassium on the multiplication of X. campestris pv. malvacearum in cotton leaf tissue.

MATERIALS AND METHODS

Cotton plants cv. Deltapine 61 were grown in a nutrient culture in 20 L black polyethylene pots in a glasshouse. The daily temperature varied between 20-32°C. Additional heating was provided during winter months to maintain nightly temperatures at 18-20°C or above. The polyethylene pots had lids with four holes. Cotton seedlings were planted through the holes and supported by black polyethylene beads contained in a polyethylene collar. Plants were later supported by tape attached to cross bars of the glasshouse. The nutrient solution was aerated continuously by a compressor pump using Teflon tubing weighted by means of a glass ring attached to the ends of the air tubes.

Seedlings were raised in vermiculite in seedlings trays. Seven days after planting, seedlings were removed, the roots washed free of residual vermiculite and transferred to the nutrient solution. Three seedlings of comparable size were planted per pot. Five days later one plant was removed to obtain uniformity of plant size and vigour.

Nutrients were added at 14, 21, 28, 35 days and at half concentration every following third day. This addition of frequent small quantities of nutrients known as 'programmed nutrient addition' (Asher and Cowie, 1970) allows uninterrupted plant growth. The pH of the solution was maintained within the range 6-7.

Growth curve

Three growth curves were obtained using three different nutrient concentrations (pers. comm. Dr P. Blamey). The nutrient solution which provided unrestricted growth was used as the standard in all experiments.

Growth curves were obtained by sampling four plants five days after planting and at regular intervals (Fig. 1) over a 53 day period. The plant sample was dried at 80°C for 24 h and the dry weight determined. Curves were obtained by plotting dry weight against time (Fig. 1).

Plant inoculation

A sterile water suspension of 10^8 cells ml^{-1} of *X. campestris* pv. *malvacearum* was prepared from a 48 h culture grown on nutrient agar supplemented with 0.1% glucose (NGA). Five ml of this suspension was added to 80 ml of NG broth which was shaken on an orbital shaker at 100 OPM at 30°C. After 72 h the suspension was centrifuged at 7500 RPM for 20 min, the pellet suspended in sterile distilled water, centrifuged, the pellet resuspended and the suspension standardised to 10^8 cells^{-1}.

Expanded but not fully mature leaves of approximately the same age and size on four week old plants were infiltrated with inoculum using a gas chromatography atomiser. The atomiser was attached to an air compressor pump which delivered the inoculum at 34.5 kPa. Infiltration of inoculum had to be completed by approximately 10.30 a.m. while the stomates were open.

Sampling

Leaf disc samples of 1 cm diameter were taken from the inoculated leaf following inoculation and at regular intervals (Fig. 2) over a four

week period. The discs were washed three times in sterile distilled water, macerated in 10 ml of sterile distilled and diluted in a tenfold series. Aliquots of 0.1 ml of 3 appropriate dilutions were spread on plates of NGA. The plates were incubated at 30°C for 48 h and the number of colonies cm^{-1} leaf disc calculated.

The increase in bacterial population over 4 weeks was plotted.

Effect of nitrogen

Four nitrogen treatments were applied to plants at transplant and at each nutrient addition. They were twice the standard nitrogen treatment, half standard, quarter standard or eighth standard.

Effect of phosphorus

The phosphorus treatments were applied at twice the standard phosphorus treatment, half standard, quarter standard and eighth standard as in the nitrogen treatments.

Effect of potassium

The potassium treatments were applied at twice standard potassium treatment, half standard, quarter standard and eighth standard as in the nitrogen treatments.

RESULTS

Growth curve

The most concentrated nutrient solution permitted unrestricted growth over the growth period required for the experiment (Fig. 1). This nutrient solution was used as the standard for all experiments. The other two nutrient solutions restricted growth on about day 36 or 43 (Fig. 1).

Effect of nitrogen

The growth rate of X. campestris pv. malvacearum in plants grown in double and half standard nitrogen concentrations was not significantly different from the standard (Fig. 2). Where the nitrogen treatment was reduced to quarter and eighth standard concentration, the population of the pathogen in both treatments differed significantly from that in the standard treatment on the fourth day, and on the 15th day in the eighth standard treatment (Fig. 3). However, disease symptoms appeared approximately 10 days after inoculation with all treatments and by day 18, no difference was detected in population number of the pathogen.

Effect of phosphorus

The growth rate of X. campestris pv. malvacearum in the different phosphorus treatments did not differ significantly from that in the standard treatment over the 25 days of the experiment (Fig. 4 and 5).

Effect of potassium

The growth of X. campestris pv. malvacearum in the potassium treatments did not differ significantly from that in the standard treatment (Fig. 6 and 7). Although the population increase in the eighth

standard treatment appeared less than in the quarter and standard treatments the population counts did not differ significantly (Fig. 7).

DISCUSSION

The growth rate of X. campestris pv. malvacearum over 25 days was unaffected by high or low concentrations of nitrogen, phosphorus and potassium. Although there were significant differences in population size between the eighth standard and standard nitrogen treatment at two sampling periods this was probably due to experimental variability in population counts rather than a reflection of real differences in growth rate. This variation could possibly be attributed to poor stomatal opening at the time of inoculation thus reducing the initial number of bacteria in the substomatal cavity. Results indicate that the nutritional status of these elements in the plant did not affect the growth rate of the pathogen and thus the development of bacterial blight. Observation of disease severity in field trials with different nitrogen treatments also failed to detect a relationship between disease severity and a particular nitrogen treatment.

Salgado and Balmer (1975) examined the response of leaves of varying physiological stages to nitrogen, phosphorus and potassium and found that the upper leaves were more susceptible than the middle or lower ones to X. campestris pv. malvacearum and that symptoms were fewer with low nitrogen and phosphorus. In a later experiment Salgado et al (1984) found a correlation between lesion size and the percentage of nitrogen in the upper and lower leaves. Our experiments were carried out on leaves at approximately the same physiological age and so our findings differed.

Debudding experiments carried out by Hall (1951) showed a slight increase in carbohydrates in the leaves with a decrease in nitrogen and Bird (1955) pointed out that plant susceptibility to X. campestris pv. malvacearum increased with a decrease in nitrogen when the carbohydrate level remained constant. Bird and Joham (1959) found that increased nitrogen increased resistance which was in contrast to previous work where Findley (1928) demonstrated side dressings of ammonium sulphate increased susceptibility. The debudding experiments provide different plant conditions for the development of bacterial blight than those under which the current experiments were carried out. However, because the conflicting results, of these experiments as well as those reported here with Salgado et al (1984) it is difficult to conclude the true effect nutritional status of the plant has on the rate of development or severity of bacterial blight. Considerable work may still need to be carried out to resolve this problem.

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ACKNOWLEDGEMENTS

I would like to express my appreciation to the Australian Cotton Research Council for their financial support for these investigations. I would like also to thank Dr P. Blamey and other members of the Department of Agriculture, University of Queensland for their help and guidance in setting up the nutrient culture.

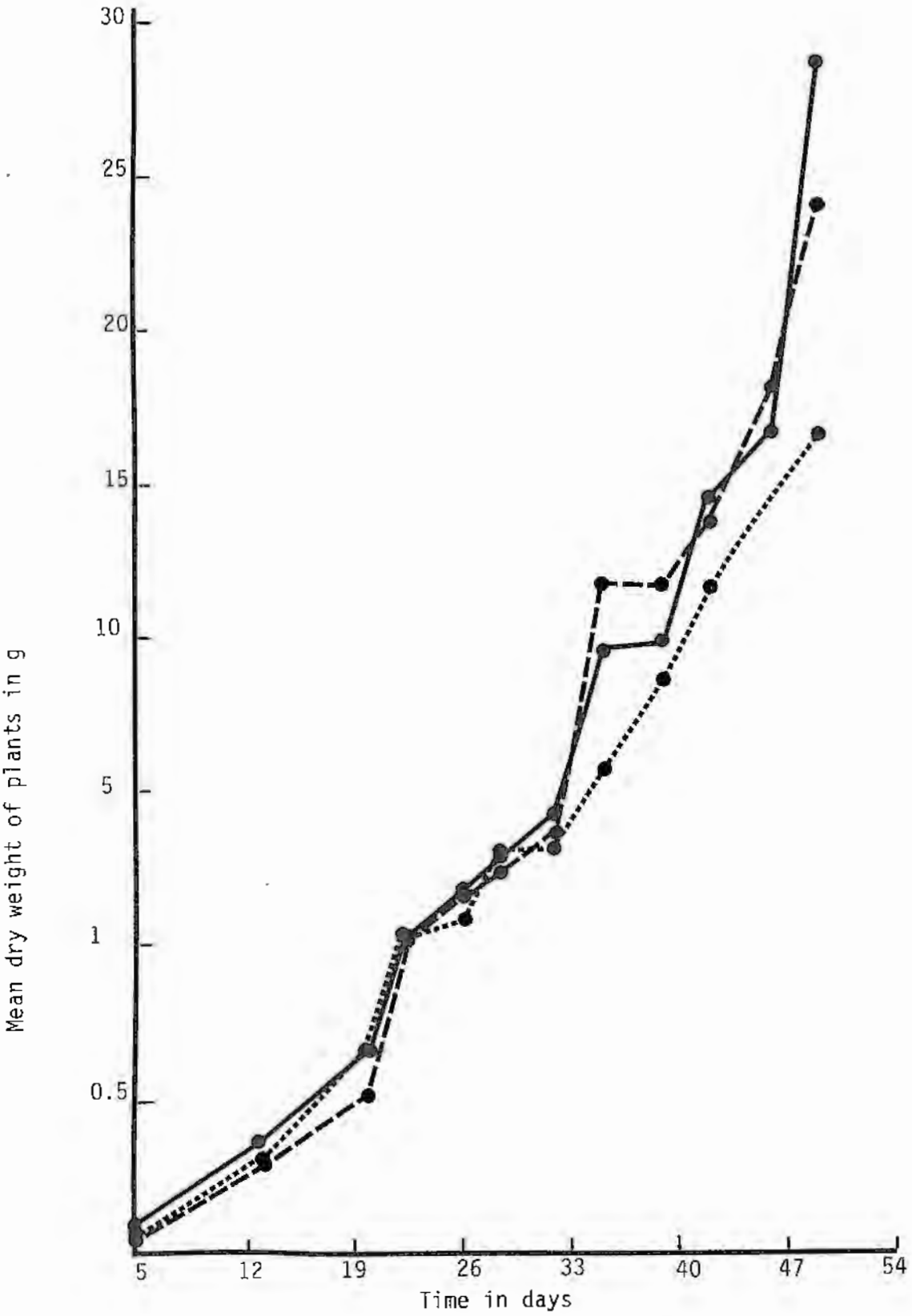

Fig. 1. Growth curves of cotton plants grown in nutrient culture under three nutrient regimes; ●——● twice standard concentration, ●– – –● standard, ●·····● half standard concentration.

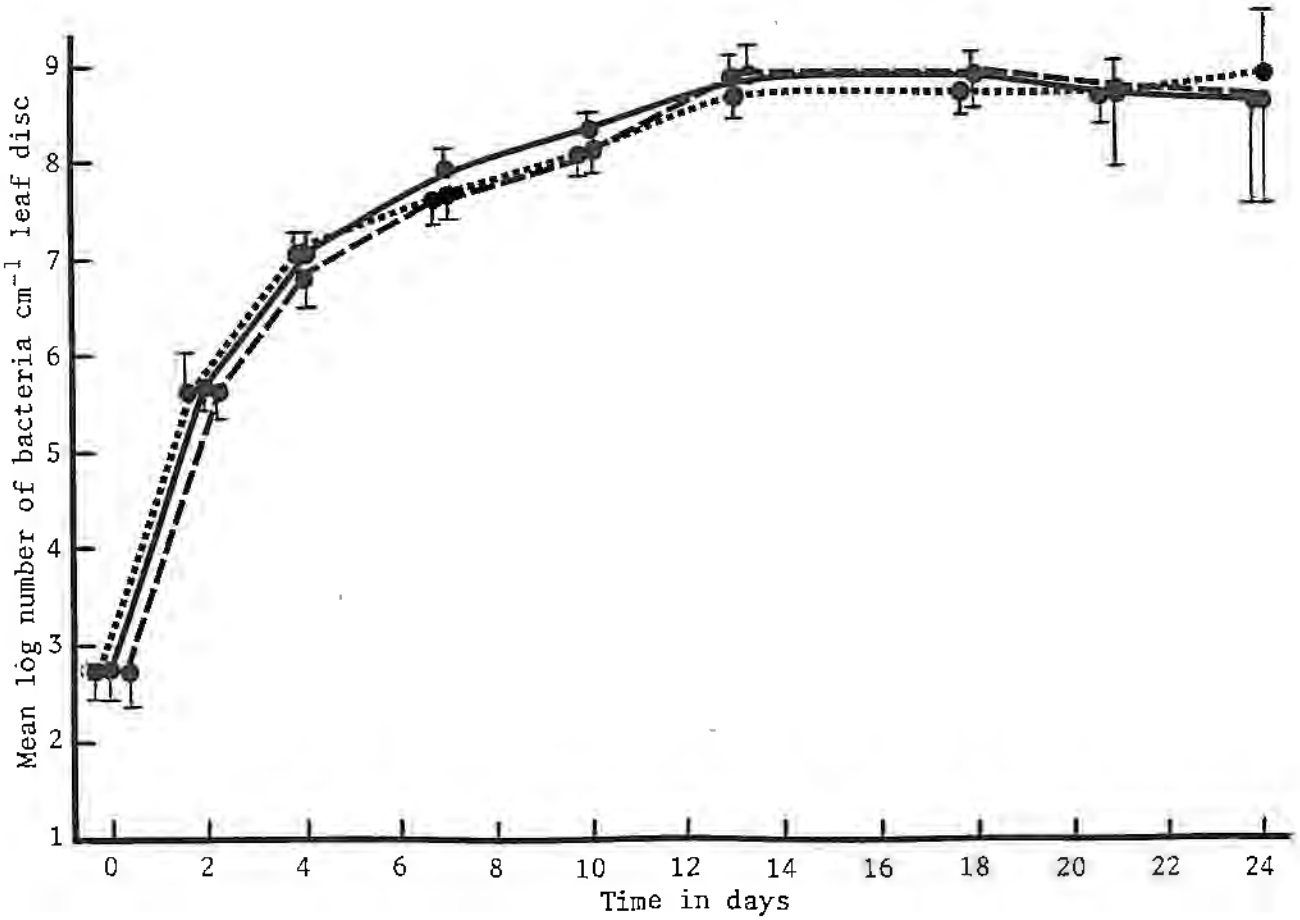

Fig. 2. Multiplication of Xanthomonas campestris pv. malvacearum in cotton grown in culture solution under three nitrogen concentration regimes; ●——● Double standard concentration ●– – –● standard concentration ●·····● half standard concentration. Vertical lines represent standard deviations from six replications.

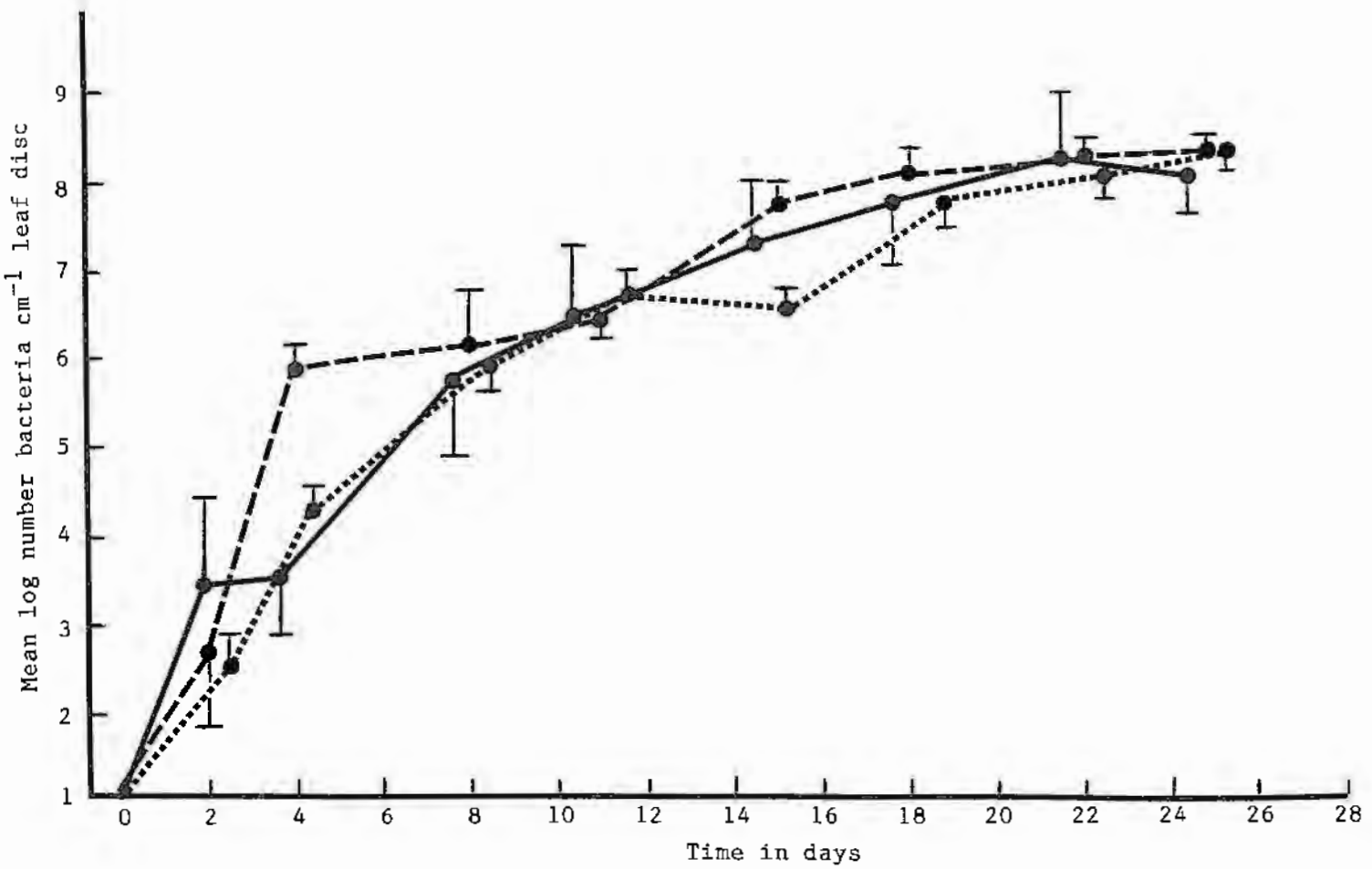

Fig. 3. Multiplication of Xanthomonas campestris pv. malvacearum in cotton grown in culture solution under three nitrogen concentration regimes; ●– – –● standard ●——● quarter standard ●·····● eighth standard concentration. Vertical lines represent standard deviations derived from six replications.

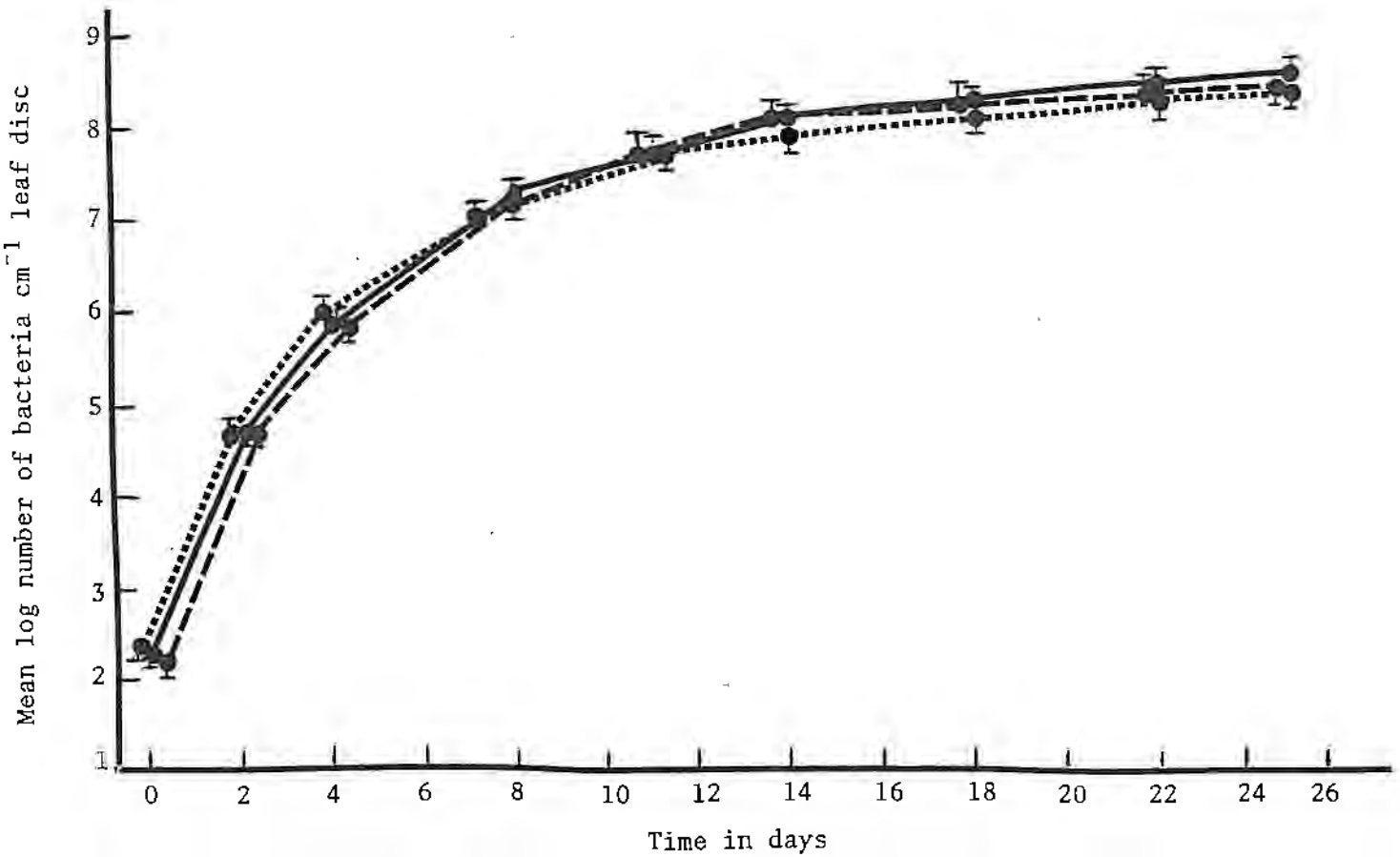

Fig. 4. Multiplication of Xanthomonas campestris pv. malvacearum in cotton grown in culture solution under three phosphorus concentration regimes; ●——● Double standard concentration ●– – –● standard concentration ●·····● half standard concentration. Vertical lines represent standard deviations from six replications.

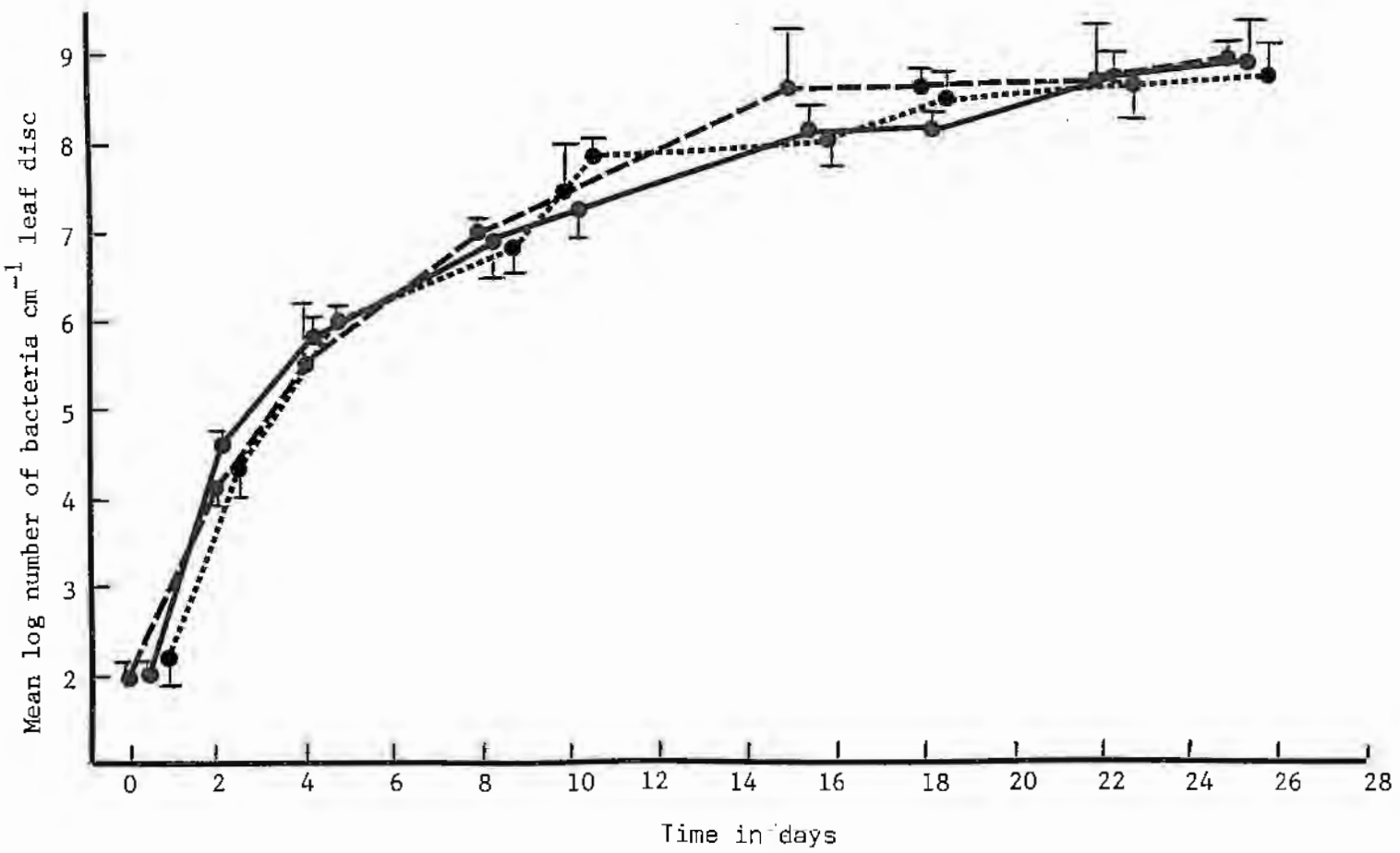

Fig. 5. Multiplication of Xanthomonas campestris pv. malvacearum in cotton grown in culture solution under three phosphorus concentration regimes. ●– – –● standard ●——● quarter standard ●·····● eighth standard concentration. Vertical lines represent standard deviations from six replications.

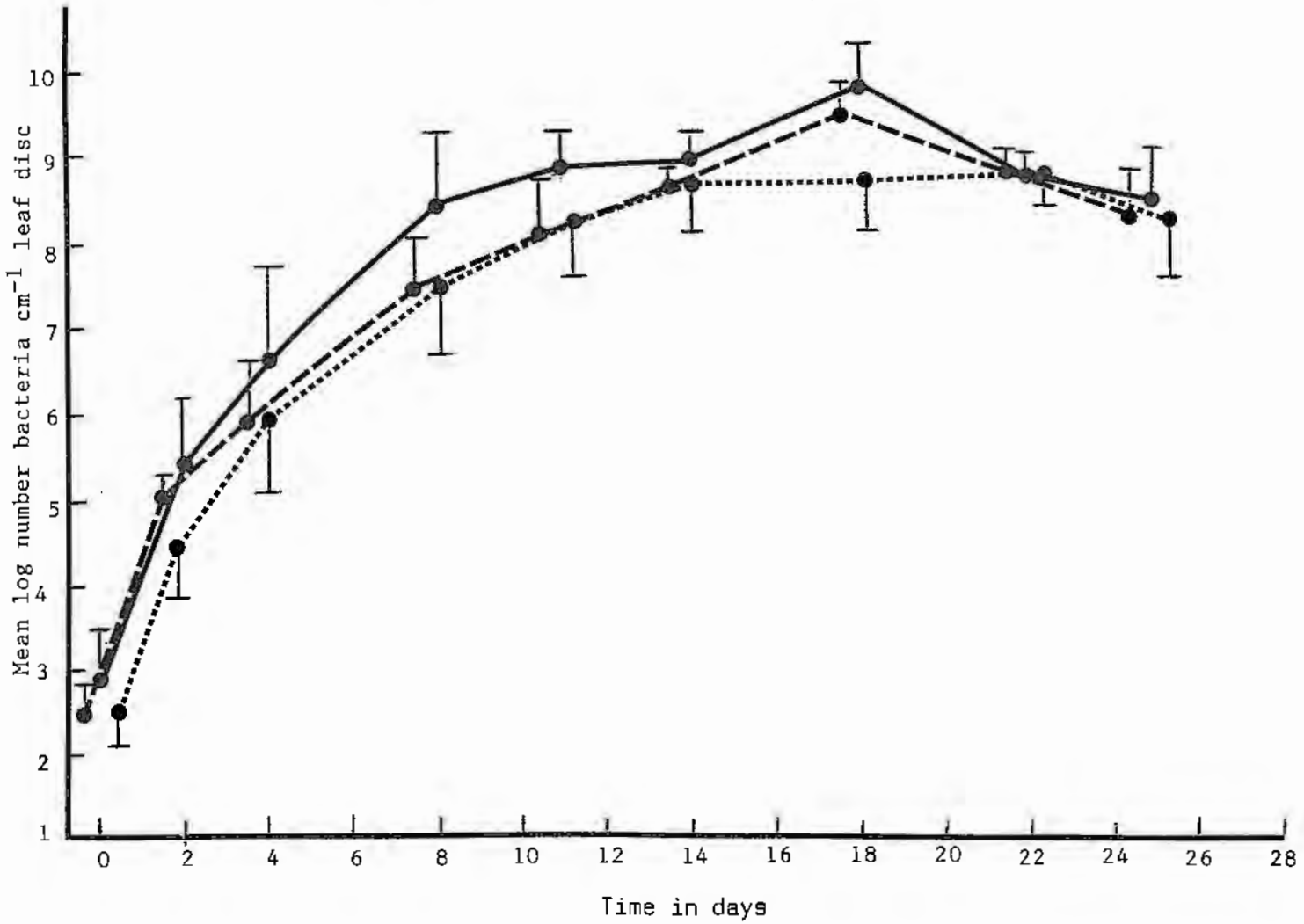

Fig. 6. Multiplication of Xanthomonas campestris pv. malvacearum in cotton grown in culture solution under three potassium concentration regimes; ●——● Double standard ●– – –● standard ●·····● half standard concentration. Vertical lines represent standard deviations derived from six replications.

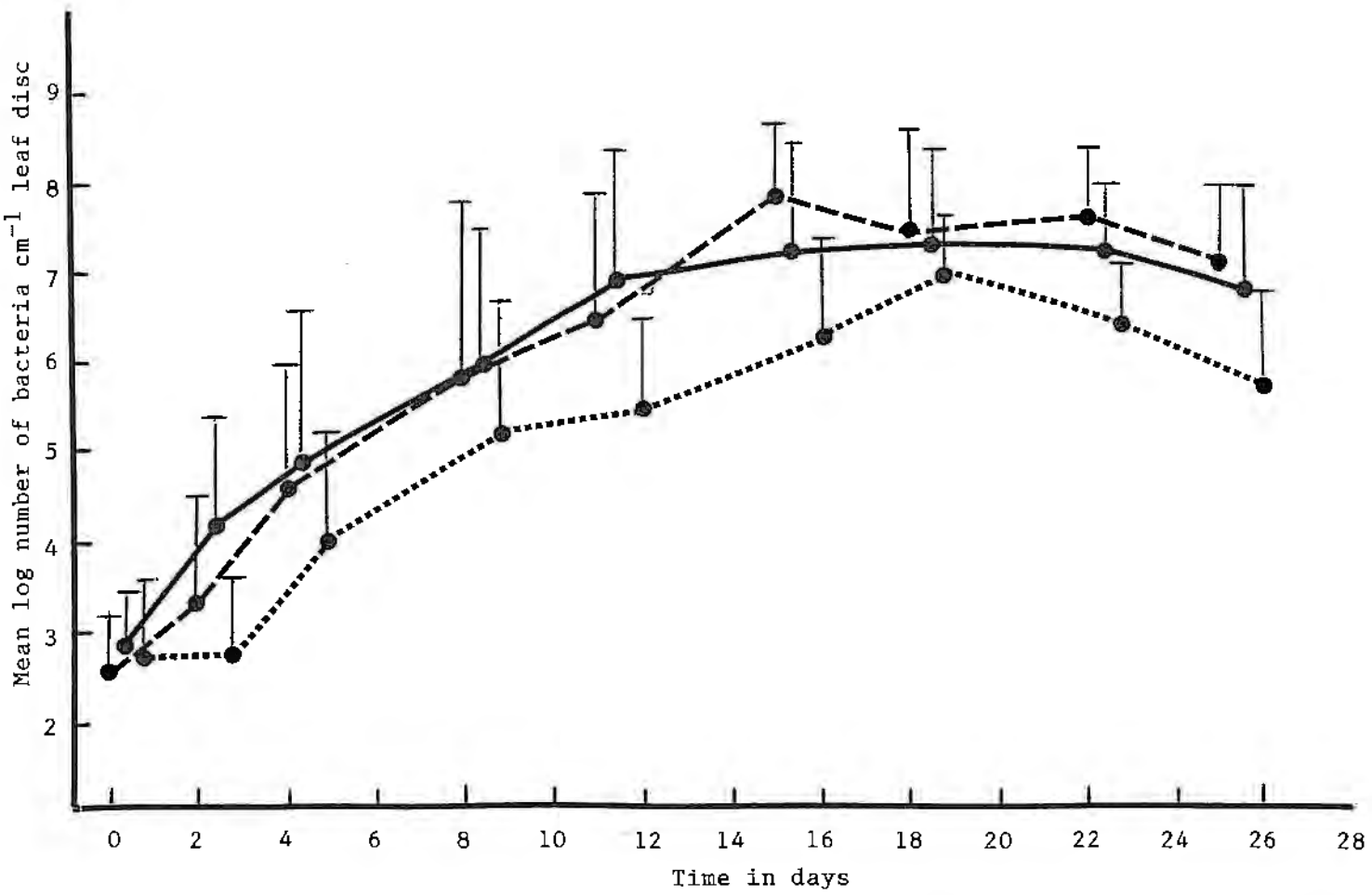

Fig. 7. Multiplication of Xanthomonas campestris pv. malvacearum in cotton grown in culture solution under three potassium concentration regimes; ●– – –● standard ●——● quarter standard ●·····● eighth standard concentration. Vertical lines represent standard deviations derived from six replications.