In vitro Inoculation Test for Resistance to Crown Gall Disease on Roses

L. Zhou and H. Fukui
Faculty of Agriculture, Gifu University, 1-1, Yanagido, Gifu, Gifu Pref., 501-11

S. Matsumoto
Faculty of Education, Gifu University, 1-1, Yanagido, Gifu, Gifu Pref., 501-11

***Rosa* 'Fashion Parade', *R. canina*, *R. canina* 'Superbe', *R.* 'Pekcougel', Anna® hybrid tea rose, and *R.* 'Meihartfo', Kalinca® floribunda rose (*syn. R.* 'Pink Wonder') were cultured by shoot tip culture and were micropropagated every 6 weeks in vitro. *Agrobacterium tumefaciens* was isolated from crown gall collected from a rose plant. The shoots inoculated with *A. tumefaciens* formed white or white-green crown galls. Four methods were used for inoculation: (1) needle prick inoculation (needle), (2) spread inoculation at upper end of shoot (upper), (3) spread inoculation at lower end of shoot (lower), (4) slice off bark and inoculate (slice). Shoot growth was not affected by inoculation of *A. tumefaciens* except for slice. Four weeks after needle inoculation, the rate of shoots which formed crown gall rose to 70% and remained stable. Therefore, the needle prick inoculation was the most successful and easiest to administer.**

Five roses: *R.* 'Fashion Parade', *R. canina*, *R. canina* 'Superbe', *R.* 'Pekcougel', Anna® hybrid tea rose, and *R.* 'Meihartfo', Kalinca® floribunda rose were inoculated by the needle method. *Rosa canina* and 'Fashion Parade' had no resistance to infection and disease. *Rosa canina* 'Superbe' was resistant to infection but lacked resistance to disease. *Rosa* 'Pekcougel', Anna® hybrid tea rose, and *R.* 'Meihartfo', Kalinca® floribunda rose (syn. *R.* 'Pink Wonder') were not infected and resisted disease infection.

INTRODUCTION

Crown gall disease is a soil-borne disease caused by *A. tumefaciens* and damages dicotyledonous plants, especially fruit trees and ornamentals. This disease is difficult to control with chemicals. Using resistant rootstocks is a good method for the control of crown gall disease, and some resistance rootstocks have been selected for roses (Boelema, 1969). However, the in vivo inoculation test for selection of resistant rootstocks to crown gall disease is affected by climate, soil conditions, and plant growth. Recently, there has been increased research on shoot-tip culture and micropropagation of *Rosa*. Therefore, we tried, in this study, to test the in vitro inoculation of micropropagated shoots and resistance to crown gall in five rose taxa.

MATERIALS AND METHODS

Plant Materials. The following rose species and cultivars were used: *R.* 'Fashion Parade', *R. canina*, *R. canina* 'Superbe', *R.* 'Pekcougel', Anna® hybrid tea rose, and

R. 'Meihartfo', Kalinca® floribunda rose (syn. *R.* 'Pink Wonder'). These cultivars were cultured by shoot-tip culture and were micropropagated every 6 weeks in vitro. The medium for shoot-tip culture and micropropagation of these roses was Murashige and Skoog's medium containing 3% sucrose and 0.2% Gelrite and adjusted to a pH of 5.7. The concentrations of 6-benzylaminopurine (BAP) and gibberellin A_3 (GA_3) were selected at the most suitable concentration for growth of each cultivar from previous experiments (Table 1). Cultures were kept at 25C with a 16-h light period for 6 weeks under 3000 lux.

Table 1. Culture condition for micropropagation of rose species and cultivars.

Rose species and culitvar	BAP concentration	GA_3 concentration
Rosa 'Fashion Parade'	1.0×10^{-5} M	1.0×10^{-6} M
R. canina	1.0×10^{-6} M	-
R. canina 'Superbe'	1.0×10^{-5} M	1.0×10^{-7} M
R. 'Pekcougel', Anna® hybrid tea rose	1.0×10^{-5} M	-
R. 'Meihartfo', Kalinca® floribunda rose	1.0×10^{-5} M	-

Pathogenic Bacteria. Crown gall was isolated from a rose plant growing in a glasshouse. *Agrobacterium tumefaciens* was isolated by culturing on Brisbane and Kerr's medium (Brisbane and Kerr, 1983) and was cultured on YEB medium containing 5 g litre^{-1} Bacto beef extract, 1 g litre^{-1} Bacto yeast extract, 5 g litre^{-1} peptone, 5 g litre^{-1} sucrose, 0.493 g litre^{-1} magnesium sulphate heptahydrate and 15 g litre^{-1} agar, and adjusted to pH 7.2.

Experiment 1. Comparison of Inoculation Methods for In VitroInoculation Test. In this experiment, the miniature rose 'Fashion Parade' was subcultured for 6 weeks and inoculated with *A. tumefaciens* cultured for 24 h on YEB medium. Four methods were used for inoculation: (1) needle prick inoculation (needle), (2) spread inoculation at upper end of shoot (upper), (3) spread inoculation at lower end of shoot (lower), (4) slice off bark and inoculate (slice).

Experiment 2. Resistance to Crown Gall Disease. Five roses: *R.* 'Fashion Parade', *R. canina*, *R. canina* 'Superbe', *R.* 'Pekcougel', Anna® hybrid tea rose, and *R.* 'Meihartfo', Kalinca® floribunda rose, were inoculated by the needle method.

RESULTS AND DISCUSSION

Experiment 1. Comparison of Inoculation Methods for the In Vitro Inoculation Test. The shoots inoculated with *A. tumefaciens* formed white or white-green crown galls (Fig. 1). Fig. 2 shows the rate of shoots forming crown gall by the four inoculation methods. The rates of infection by the needle and lower methods reached 45% after 2 weeks and were higher than those using upper and slice. Inoculation by needle and lower, therefore, stimulated infection of *A. tumefaciens*.

Four weeks after inoculation, the rate using the needle method, rose to 70% and remained stable. That by slice increased rapidly and continued to rise until the 6th week. The rates by the lower and upper methods leveled out at about 50%.

For the in vitro inoculation test, a stable rate of infection has to be established earlier. Although slice indicated a high rate after 6 weeks inoculation, the rate continued to increase. Therefore, the rate will not be constant. With the needle method, infection reached 70% after 4 weeks and then remained stable and we consider this the best method.

Figure 1. Shoots inoculated with *Agrobacterium tumefaciens* formed white or white-green crown galls.

Shoot growth was not affected by inoculation with *A. tumefaciens* except under the slice method. Growth of shoots inoculated by slice, declined as the crown gall increased, and the shoots turned brown.

The resistance to crown gall disease on roses has been measured by in vivo testing (Boelema, 1969; Brown, 1923). The results of this in vivo test method are affected by the stage of growth and the environment, i.e. temperature, humidity, solar radiation, soil condition and watering, and are not constant.

Recently, the resistance to some diseases has been tested in vitro, in tomato (Toyoda et al., 1988), potato (Nakahara et al., 1990), and tobacco (Chatani et al., 1994). These results are constant, because the growth of the plants in vitro is not affected by environmental conditions, also we are able to use plants in the same growth stage for the test.

There have already been many practical reports on in vitro propagation of roses, and the needle prick inoculation method in this paper was good for manipulation and sensitivity. Therefore, this in vitro inoculation method will become an important method for the selection of resistant rootstocks to crown gall disease.

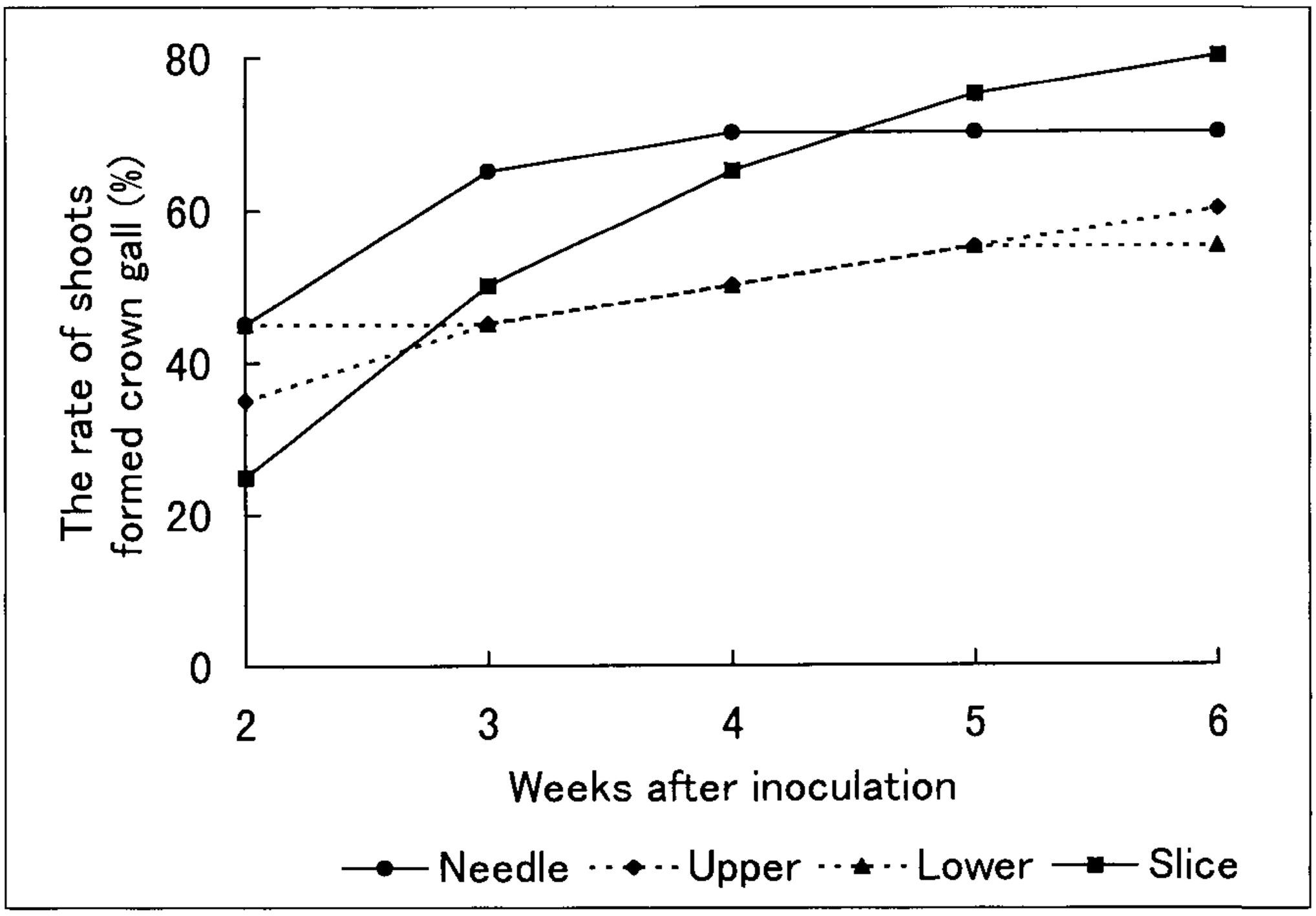

Figure 2. The rate at which shoots formed crown gall, comparing four inoculation methods.

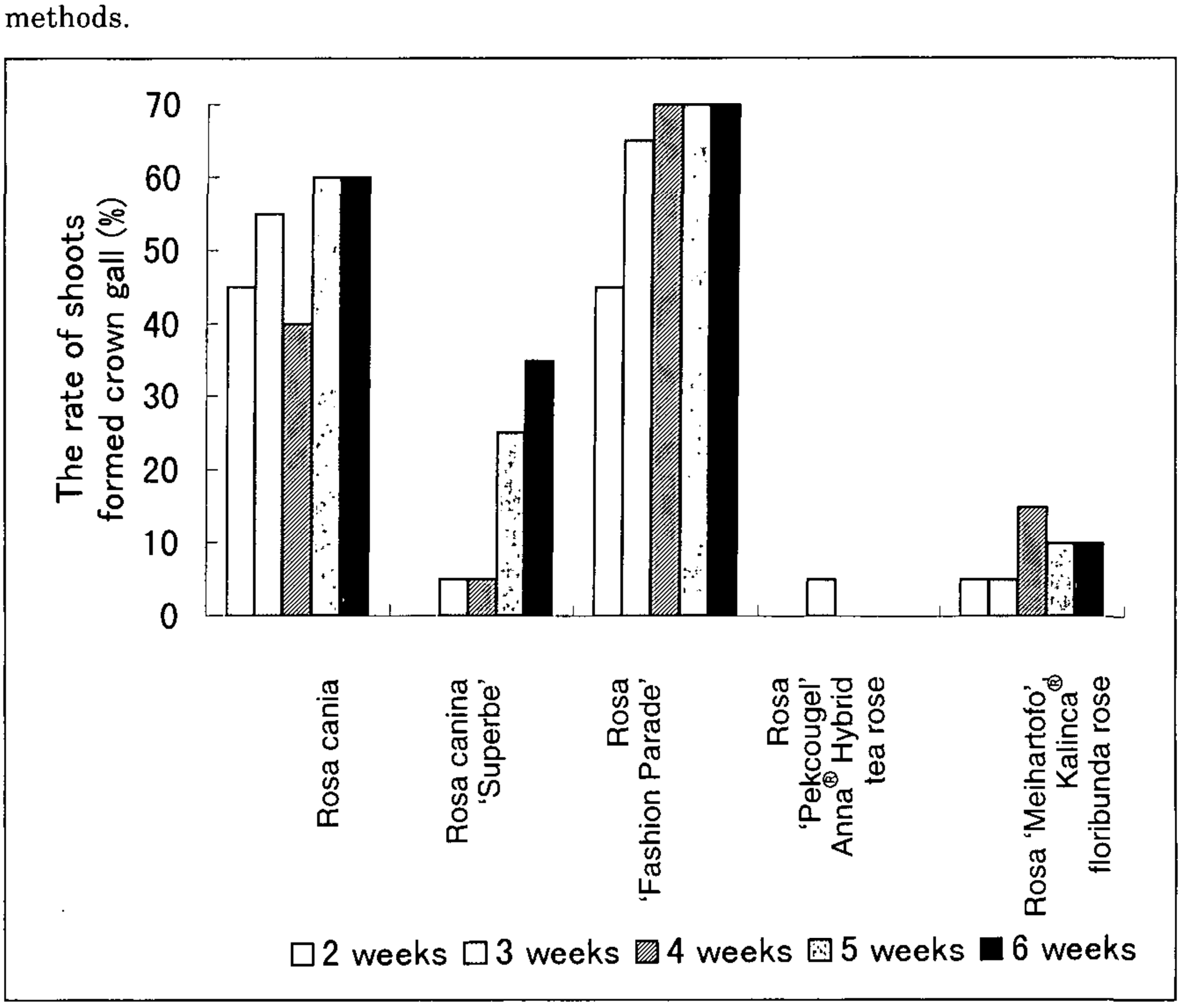

Figure 3. The rate at which shoots formed crown gall in the five roses.

Experiment 2. Resistance of Rose Species and Cultivars to Crown Gall Disease. Two weeks after inoculation 45% of *R. canina* and 'Fashion Parade' were already infected (Fig.3). Thereafter the rate of plants forming crown gall increased slightly and reached between 60% and 70%. *Rosa canina* 'Superbe', a cultivar selected from *R. canina* for rose rootstocks, showed no infection 2 weeks after inoculation. The infection rate was low until the 4th week, but thereafter shoots forming crown gall increased to 40% after 6 weeks. *Rosa* 'Pekcougel', Anna® hybrid tea rose, and *R.*'Meihartfo', Kalinca® floribunda rose showed little sign of infection.

From the high rate of infection in *R. canina* and 'Fashion Parade', we decided that these two roses had no resistance to infection and disease development. The low rate 4 weeks after inoculation in *R. canina* 'Superbe' indicated that this rootstock cultivar had resistance to infection. But, the increase after 5 weeks and over, indicated a lack of resistance to disease development in this cultivar. *Rosa* 'Pekcougel', Anna® hybrid tea rose, and *R.*'Meihartfo', Kalinca® floribunda rose had resistance to infection.

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