Appendix A Analysis of resource- consumer model

Model details. Neighboring *A. tumefaciens* lineages compete for a variety of resources. When in the vicinity of a crown gall, virulent pTi$^+$ cells have access to opine resources that avirulent pTi$^-$ cells are unable to metabolize. The suite of opines released by infected plants varies considerably and depends on the type of Ti plasmid genetically transforming the plant (Dessaux et al. 1998). In this paper, we focus on octopine-type Ti plasmids whose T-DNA promotes plant production of several related opines (octopine-type opines and mannityl opines, that are derived from amino acids and simple organic acids or sugars) which can serve as nutritional sources of carbon and/ or nitrogen for octopine-type pTi$^+$ cells (Petit et al. 1983; Dessaux et al. 1998). Previous research suggests that for most octopine-type pTi$^+$ cells, octopine is a more efficient source of nitrogen than it is for carbon (Bell 1990). To simplify discussion of the model we assume that nitrogen is limiting, though the presented model can alternatively be interpreted to represent opines acting as a source of carbon under carbon-limiting conditions.

Our model builds on the classic models of microbial dynamics of Monod (1949) and Tilman (1980; Tilman et al. 1982) to describe the population dynamics of pTi$^+$ and pTi$^-$ cells competing for two potentially limiting but substitutable resources: nitrogen-containing opines and other nitrogen sources. Our model explicitly tracks resource dynamics and assumes that all competitive interactions are mediated by limiting resources. The population growth rate of pTi$^+$ cells is determined by the relative balance of the strain's birth rate, which depends on the harvest and utilization of both opines (O) and non-opine nitrogen sources (A), and its death rate, which occurs at a constant per capita rate (m_{pTi+} ; see Table 2 for complete description of parameters).

$$\frac{dN_{pTi+}}{dt} = \left(\frac{u^A_{pTi+} e^A_{pTi+} A + u^O_{pTi+} e^O_{pTi+} O}{1 + e^A_{pTi+} h^A_{pTi+} A + e^O_{pTi+} h^O_{pTi+} O} \right) N_{pTi+} - m_{pTi+} N_{pTi+}$$

Similarly, the population growth rate of cells lacking the Ti plasmid also depends on its relative birth and death rates, however the birth rate of these cells depends only on the harvest and utilization of non-opine nitrogen sources since it cannot catabolize opines.

$$\frac{dN_{pTi-}}{dt} = \left(\frac{u_{pTi-}^{A} e_{pTi-}^{A} A}{1 + e_{pTi-}^{A} h_{pTi-}^{A} A} \right) N_{pTi-} - m_{pTi-} N_{pTi-}$$

Plants manifesting crown gall disease typically release a suite of opine molecules (Dessaux et al. 1998) and there are likely to be several additional forms of non-opine nitrogen sources in the rhizosphere (Nannipieri and Eldor 2009). For simplicity and practicality, we describe the amount of these classes of resources by aggregate terms (e.g. *O* and *A*). More realistically, competition will depend on the resource dynamics of many more substrates that agrobacterial cells may utilize, encounter, or handle differently. The rate at which opine levels change is determined by the relative balance of the rate at which opines are supplied to the environment and the rate at which pTi$^+$ cells catabolize them. We assume that both types of resources are flowing into and out of the environment at a fixed rate (D_O and D_A). Nutrients flow into the environment at a constant concentration (O_0 and A_0), flow out of the environment at their present concentration in the environment (*O* and *A*), and are not recycled following cell death.

$$\frac{dO}{dt} = D_O(O_0 - O) - \left(\frac{e_{pTi+}^{O} O}{1 + e_{pTi+}^{A} h_{pTi+}^{A} A + e_{pTi+}^{O} h_{pTi+}^{O} O} \right) N_{pTi+}$$

Changes in environmental levels of non-opine nitrogen sources also reflect the relative balance of the rate at which they are supplied to the environment and the rate at which they are consumed. However, unlike opines they can be consumed by both pTi$^+$ and pTi$^-$ cells.

$$\frac{dA}{dt} = D_A(A_0 - A) - \left(\frac{e^A_{pTi+} A}{1 + e^A_{pTi+} h^A_{pTi+} A + e^O_{pTi+} h^O_{pTi+} O} \right) N_{pTi+} - \left(\frac{e^A_{pTi-} A}{1 + e^A_{pTi-} h^A_{pTi-} A} \right) N_{pTi-}$$

The model assumes that the substitutable resources co-occur such that the harvest rate of pTi$^+$ cells can be described by the two-resource extension of Holling's disc equation (Holling 1959; Vincent et al. 1996). In order to isolate the effects of resource competition on agrobacterial population dynamics, at present our model does not allow for the horizontal transmission of the Ti plasmid, however opines are known to stimulate conjugation of the plasmid (Kerr et al. 1977; Farrand 1998). The importance of conjugation to the fitness of the Ti plasmid in the disease environment is itself an interesting issue for future theoretical and empirical investigation.

Conditions for coexistence. Coexistence of pTi$^+$ and pTi$^-$ bacteria is possible if 1) the slope of the pTi$^+$ consumption vector is greater than that of the pTi$^-$ consumption vector; and 2) the minimum non-opine nitrogen source supply of pTi$^+$ cells is greater than that of pTi$^-$ cells ($A^*_{pTi-} < A^*_{pTi+}$) (Vincent et al. 1996). This first condition is always true provided that opines are present in the environment, since pTi$^-$ cells are not able to consume opines, while pTi$^+$ cells can consume opines (Figure 2). pTi$^+$ cells require more non-opine nitrogen supply than pTi$^-$ cells when $\frac{m_{pTi-}}{e^A_{pTi-}(u^A_{pTi-} - m_{pTi-} h^A_{pTi-})} < \frac{m_{pTi+}}{e^A_{pTi+}(u^A_{pTi+} - m_{pTi+} h^A_{pTi+})}$ (Equation 2). There are four ways this can happen: 1) pTi$^+$ cells have a higher mortality rate ($m_{pTi+} > m_{pTi-}$); 2) pTi$^-$ cells are more efficient at utilizing non-opine nitrogen sources ($u^A_{pTi-} > u^A_{pTi+}$); 3) pTi$^+$ cells have a greater handling time of non-opine nitrogen sources ($h^A_{pTi+} > h^A_{pTi-}$); or 4) pTi$^-$ cells are more likely to encounter and accept these nutrients ($e^A_{pTi-} > e^A_{pTi+}$). We have experimentally demonstrated that there is a fitness cost associated with harboring the Ti plasmid under resource liming conditions (Platt et al. 2011), suggesting that the inequality in Equation 2 is true; however we do not currently know which parameter(s) underlies this cost. Most likely, the cost is a result of differences in mortality rates or utilization efficiencies; however this remains an issue for future work.

Resource competition and population size. A population of pTi$^+$ cells growing in a nitrogen-limited environment supplied with both opine and non-opine nitrogen sustains an equilibrium population size that depends on the supply rates of both types of resources (Figure 3):

$$N^*_{pTi+} = [D_A(A_0 - A^*] + D_O(O_0 - O^*)] * \left[\frac{1 + e^A_{pTi+} h^A_{pTi+} A^* + e^O_{pTi+} h^O_{pTi+} O^*}{e^A_{pTi+} A^* + e^O_{pTi+} O^*}\right]$$

In contrast, because pTi$^-$ cells cannot catabolize opine nitrogen resources, the equilibrium population size of a pTi$^-$ population depends only on the supply rate of non-opine nitrogen sources (Figure 3):

$$N^*_{pTi-} = [D_A(A_0 - A^*)] * \left[\frac{1 + e^A_{pTi-} h^A_{pTi-} A^*}{e^A_{pTi-} A^*}\right]$$

When both consumers are present, the equilibrium population sizes of pTi$^+$ and pTi$^-$ cells partially depend on the degree to which the other strain consumes the resources making them effectively unavailable:

Equation 3

$$N^*_{pTi+} = \left[D_A(A_0 - A^*) - N^*_{pTi-} \left[\frac{e^A_{pTi-} A^*}{1 + e^A_{pTi-} h^A_{pTi-} A^*} \right] \right] * \left[1 + e^A_{pTi+} h^A_{pTi+} A^* + e^O_{pTi+} h^O_{pTi+} O^* \right] * \left[\frac{1}{e^A_{pTi+} A} \right]$$

Equation 4

$$N^*_{pTi-} = \left[D_A(A_0 - A^*) - N^*_{pTi+} \left[\frac{e^A_{pTi+} A^*}{1 + e^A_{pTi+} h^A_{pTi+} A^* + e^O_{pTi+} h^O_{pTi+} O^*} \right] \right] * \left[1 + e^A_{pTi-} h^A_{pTi-} A^* \right] * \left[\frac{1}{e^A_{pTi-} A^*} \right]$$

From Equation 3 we identified that the equilibrium population size of pTi$^+$ cells increases linearly with increasing opine supply. In contrast, the equilibrium population size of pTi$^-$ cells does not depend on opine supply, but is predicted to decline when opine availability is high due to resultant large populations of pTi$^+$ cells removing non-opine nitrogen sources from the environmental supply (see Equation 4).

Appendix B Costs and benefits of pTiR10

In order to demonstrate that the costs and benefits associated with the Ti plasmid that we have demonstrated are not specific to the strain we are working with (*A. tumefaciens* 15955) we tested our model's predictions using *A. tumefaciens* R10 which harbors an octopine-type Ti plasmid. We competed this strain against a kanamycin resistant derivative strain cured of pTiR10 (KYC55) under environmental conditions containing octopine (6.5 mM) or lacking this resource. KYC55 and R10 are demonstrably not as isogenic as the 15955/TP101 pair used for the bulk of our studies (Cho et al. 1997). Consistent with our previous results we observed that pTi^+ R10 cells are at a competitive disadvantage to pTi^- KYC55 cells when octopine is absent from the environment (Figure A1, $t = -2.2$, 12 df, $p < 0.05$). Also as predicted, octopine availability significantly increased the fitness of cells harboring the virulence plasmid (Figure A1, $F_{1,12} = 6.71$, $p < 0.05$). For this experiment the media contained AT minimal media salts, 500 µM phosphate, and was buffered by MES to pH 5.6. Under these conditions carbon availability limits population growth. We differentiated strains by plating onto media that either lacked or contained 150 µg/ml kanamycin.

We also observed that opine availability increased the carrying capacity of pTi^+ R10 populations ($t = -4.25$, df = 7, $p < 0.01$), but did not affect the carrying capacity of pTi^- KYC55 populations (Figure A2, $t = -2.16$, df = 11, ns).

Figure A1. *A. tumefaciens* R10 cells harboring pTiR10 had a competitive advantage over pTi cured derivatives when octopine was present, but were at a competitive disadvantage when octopine was absent. Values represent mean ± standard error of eight replicates.

Figure A1

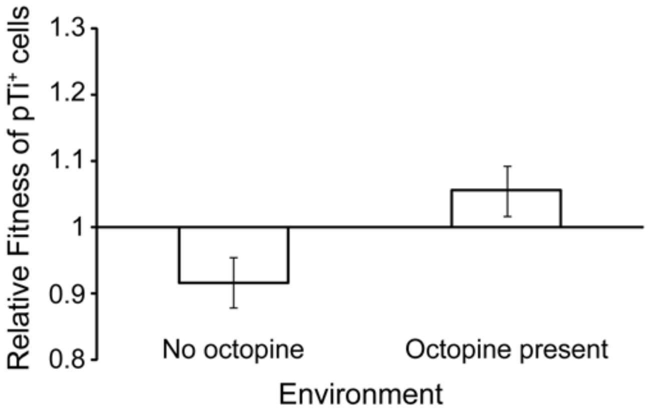
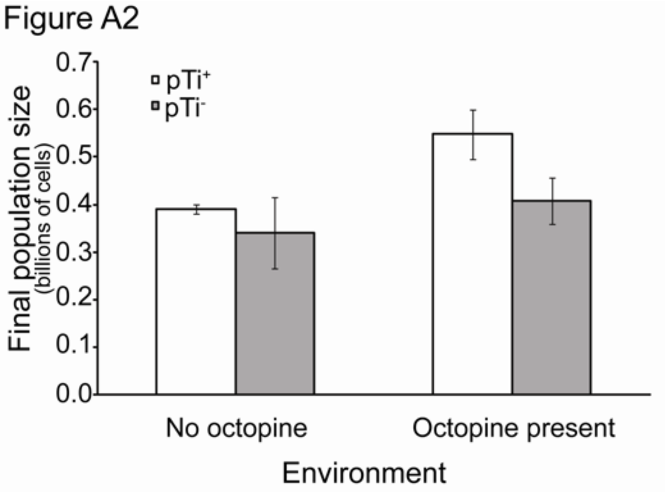


Figure A2. The carrying capacity of the pTi^{+} (R10), but not the pTi^{-} (KYC55), population increased with opine availability. Values represent mean ± standard error of eight replicates.



Appendix C Population elasticity in response to opine availability

A. tumefaciens strain 15955 achieved higher population density in response to being supplied with higher levels of octopine as the sole source of nitrogen (Figure A3A; $\beta_{[Oct]} = 1.1$, t = 13.8, p < 0.001) and as the sole source of carbon (Figure A3B; $\beta_{[Oct]} = 0.1$, t = 9.4, p < 0.001). In both cases, this effect eventually saturated, suggesting that other factors besides nitrogen or carbon availability, respectively, became limiting (when octopine was the sole nitrogen source: $\beta_{[Oct]^2} = -0.17$, t = -8.6, p < 0.001; and when octopine was the sole carbon source: : $\beta_{[Oct]^2} = -0.002$, t = -3.6, p < 0.01). OD_{600} nm was measured 87 hours after inoculation. Values are mean ± standard error of three replicates.

Figure A3. *A. tumefaciens* 15955 population size increases with octopine availability. Prior to octopine supplementation nitrogen (A) or carbon (B) limits population growth.

Figure A3A

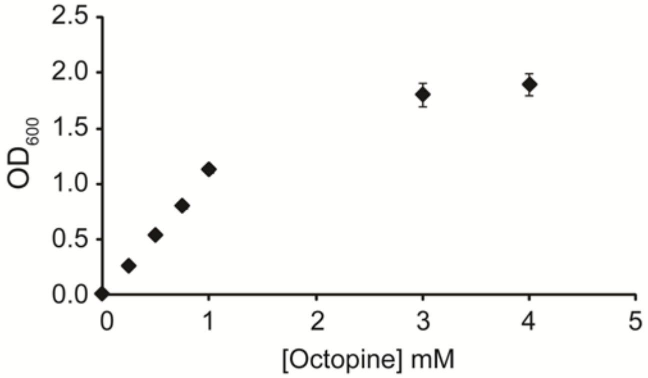


Figure A3B