I studied the effects of interactions between Nicotiana longiflora and N. plumbaginifolia on their mating systems. First, I determined interpopulational variability in traits associated with mating system (i.e. pollen:ovule ratio). Second, I determined if this variability is also found when estimating outcrossing rates and how sympathy might affect outcrossing rates. Finally, I explore the importance of post-pollination mechanisms determining offspring paternity in natural population of both Nicotiana species. Results showed significant interpopulational variability in N. longiflora floral traits but not in the selfer N. plumbaginifolia, whose seeds were primarily sired by self-fertilization. Corolla length and pollen:ovule ratio were relatively good predictors of mating system estimated as self seed set. Sympathy showed a negative effect on N. longiflora fitness and N. plumbaginifolia outcrossing rate. An increase in genetic diversity was detected on sympatric N. plumbaginifolia populations, suggesting the occurrence of hybridization with N. plumbaginifolia being the maternal parent. Outcross pollen had a greater growth rate than self-pollen in N. longiflora but not in N. plumbaginifolia; indicating the occurrence of cryptic self-incompatibility in N. longiflora. N. plumbaginifolia's offspring was equally set by self and outcross pollen in two donor crosses; however, in three-donor hand pollinations, self- and heterospecific-pollen were equally successful at siring offspring. Overall, results support that interactions between N. longiflora and N. plumbaginifolia occur in sympatric natural populations. In sympathy, asymmetrical hybridization is a possibility, but a decrease in outcrossing rates in N. plumbaginifolia and the outcross advantage in N. longiflora might act as isolation mechanisms.