Evolution of Fluconazole Resistance in *Candida albicans*

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Abstract

Candida albicans is an opportunistic fungus which develops in immunodeficient patients with varying degrees of susceptibility to fluconazole. Approximately 99% of all strains of *Candida albicans* are susceptible, but there are problematic resistant strains that are only treatable with alternative, sometimes highly toxic, antifungal medication. We modeled a system with two strains of *Candida albicans*, one susceptible and one resistant population, with ordinary differential equations to analyze the behavior of our two strains in an individual while applying varying degrees of fluconazole. In addition to the determinstic model, we created a stochastic model and conducted numerous simulations. With both models we found the amounts of antifungal treatment necessary to ensure a stable population where the two strains coexisted and the immune system could naturally keep the fungus' growth in check. Furthermore, we determined why resistant strains emerge when antifungal agents aren't administered or taken properly.

1 Introduction

C. albicans is a commensal and opportunistic human pathogen which is the cause of most fungal infections in immunocompromised patients–including HIV patients, cancer patients, organ transplant recipients, and surgery patients [1], [12]. In fact, it is estimated to infect 90% of HIV patients [4]. Early symptoms of infection are white or red patches in the throat, difficulty swallowing, and nausea [10]. If left untreated the fungal infection can spread to the lungs or digestive tract and lead to extensive bodily deterioration or even death [13].

The fungus reproduces through the asexual process of budding or by producing spores. Colonization by *C. albicans* is not always continuous but actually varies between a carriage rate of 1.0 to 80.6%. However, the reproductive rate of the organism is kept in check by physical barriers such as the skin, competition with microflora, and host defense mechanisms. Infections can be either superficial, usually on mucous membranes, or hematogenously disseminated, which results in a systemic disease that has a high mortality rate [8].

Recently a new azole called fluconazole has been used to treat *C. albicans* infections [3]. Fluconazole is an orally active, bis-triazole antifungal agent that is less toxic and has a greater in-vivo activity against *C. albicans*. Fluconazole operates by inhibiting lanosterol C-14 $\alpha - demethylase$, the cytochrome P-450 dependent enzyme essential for ergosterol synthesis of fungal cell wall, thereby disrupting the structural integrity of the plasma membrane and making it more susceptible to further damage by fluconazole [5],[3]. Fluconazole also alters the activity of membrane bound enzymes, such as those that are necessary for nutrient transport and chitin synthesis. However, studies indicate that infection recurs within about 3 months of treatment in up to 80% of HIV patients [4]. Because of this there has been a greater number of patients with *C. albicans* strains that are resistant to fluconazole. Although other azoles can be used for treatment resistance to these can develop as well [11]. Due to this trend, resistance to fluconazole and other antifungal medications has become a major clinical problem in management of immunosuppressed patients.

Resistance is usually due to natural selection of favorable genetic traits within the population. Genetic variability in *C. albicans* is assumed to be due, solely, to genetic mutations in the fungus due to the fact that there is currently no known method by which *C. albicans* may undergo sexual

recombination [9]. In general, the rate of adaptation to antifungal treatment is a combination of the rate at which genetic variability, due to mutations in the case of *C. albicans*, arises and the sequence in which mutations confer an adaptive advantage [7], [1]. It is believed that resistance in *C. albicans* to fluconazole is caused by reduced cell permeability and target site alteration [2], [6].

Resistant strains flourish when the antifungal treatments are not implemented correctly. Though different strains exist, resistant strains of *Candida albicans* can coexist with non-resistant strains. If an antifungal agent is used after the resistant strains have developed, the antifungal agent will only be capable of killing the susceptible strain, leaving behind less virulent, resistant strains.

In this study we will look at the simplest possible deterministic model and focus on the joint dynamics of susceptible and resistant strains of the fungus, Candida albicans. Resistance to antifungal treatment in a given population of *Candida albicans* is modeled via a system of nonlinear differential equations. We take into account that treatment is available and that the fungus mutates with some low probability. Using this model we will also create a stochastic model to be used to simulate the treatment of fungal infections. Using these models will determine the effect of various parameters on the development of resistance in *Candida albicans.*

2 Methodology

2.1 Deterministic Model

In the development of the deterministic model, we assume that if an antifungal agent is used after the resistant strains have developed, the antifungal agent will only be effective in killing the susceptible strain, while the resistant strain can survive, but with reduced virulence. Resistance to antifungal treatment in a given population of *Candida albicans* is modeled via a system of nonlinear differential equations. We let $x(t)$ denote the density of susceptible *Candida albicans* at time t; $y(t)$ is the density of resistant *Candida albicans* at time t. It is assumed that the dynamics are separated by two thresholds. The stable density where the fungus is resident and the immune system can regulate its population is denoted by k_1 (allele effect), while k_2 denotes the maximum density that can be regulated by the immune system.

We further assume that treatment is available at the rate b and that the fungus mutates into the non-resistant strain with probability q. The growth rate for the susceptible fungus is r_1, and r_2 for the resistant fungus. It is assumed that $r_1 > r_2$ and $k_1 < k_2$. With these assumptions we arrive at the following model.

$$\frac{dx}{dt} = r_1 x \left(1 - \frac{x}{k_1}\right)\left(1 - \frac{x+y}{k_2}\right) - bx \tag{1}$$

$$\frac{dy}{dt} = r_2 y \left(1 - \frac{y}{k_1}\right)\left(1 - \frac{x+y}{k_2}\right) + bqx \tag{2}$$

The deterministic model is visually represented by the diagram in Figure (1).

In our model, when there is no treatment ($b = 0$), we have two equilibrium points. As shown in Figure (2) there is one low level equilibrium at k_1, representing the resident population, and one take off point, k_2, where, if we perturb $x + y = k_2$ the immune system will suppress the fungus back to the low level equilibrium point or the fungus will grow exponentially.

2.1.1 Definition of Parameters

x = susceptible population of *Candida albicans*
y = resistant population of *Candida albicans*
r_1 = the maximal intrinsic reproductive rate of the susceptible strain x
r_2 = the maximal intrinsic reproductive rate of the resistant strain y
k_1 = the stable threshold where the fungus is resident when no antifungal agent is implemented
k_2 = the unstable threshold where the fungus may grow exponentially when no antifungal agent is implemented
q = the probability that a non-resistant strain of *C. albicans* will mutate into a y state, given that it has been affected by the antifungal agent
b = the rate at which non-resistant strains leave the population either from death induced by fluconazole or from mutating into the resistant strain

2.1.2 Parameter Conditions

$2k_1 < k_2$
For this model, we assume that when *Candida albicans* exists in small amounts,

there is no competition between strains, but for greater amounts, approaching the take-off point, the two strains have an additive effect. This assumption holds only if k_2 (the take-off point, without treatment) is significantly larger than k_1. In addition, for $\frac{k_2}{k_1} < 2$ we observe a nonlinear fixed point with dubious biological meaning.
$r_1 > r_2$
This assumption is based on the hypothesis that there is a trade-off of a lower reproductive rate for a higher resistance.

2.1.3 Rescaled Model

If we rescale our model such that
$\hat{x} = \frac{x}{k_1}$
$\hat{y} = \frac{y}{k_1}$
$\hat{b} = \frac{b}{r_1}$
$k = \frac{k_2}{k_1}$, $(k > 2)$
$r = \frac{r_2}{r_1}$, $(r < 1)$
$t = \tau\alpha$
our equations become:

$$\frac{d\hat{x}}{d\tau} = \hat{x}(1-\hat{x})\left(1 - \frac{\hat{x}+\hat{y}}{k}\right) - \hat{b}\hat{x} \tag{3}$$

$$\frac{d\hat{y}}{d\tau} = r\hat{y}(1-\hat{y})\left(1 - \frac{\hat{x}+\hat{y}}{k}\right) + \hat{b}q\hat{x} \tag{4}$$

Rescaling our model will be useful when we evaluate the deterministic model in Section 4. For simplicity, we drop the ˆ notation and rename τ as t in the remainder of the paper.

2.2 Stochastic Model

In order to provide a comparison for the deterministic model stochastic simulations were run using a stochastic model adapted from the deterministic model. During the simulation five different events can occur. The non-resistant x population can either increase or decrease by one while the resistant y population remains the same; the x population can remain the same while the y population can increase or decrease by one; or the x population can decrease by one while the y population increases by one.

Event		Rate Of Event	If rate
$x, y \longrightarrow$	$x+1$,y	$R(x) = x(1-x)(1-\frac{x+y}{k})$	> 0
	$x-1$,y		< 0
$x, y \longrightarrow$	x,$y+1$	$R(y) = ry(1-y)(1-\frac{x+y}{k})$	> 0
	x,$y-1$		< 0
$x, y \longrightarrow$	$x-1$,y	$R(bx) = bx$	
	$x-1$,$y+1$	$R(bqx) = bqx$	

An exponential function is used to calculate the time till the next event with respect to θ – the total rate. θ is calculated by adding the absolute value of each of the rates. Therefore $\theta = |R(x)| + |R(y)| + R(bx)$.

In order to determine the probability of each event, the absolute value of the rate of each event is divided by θ. These probabilities are arranged on a scale from 0 to 1, as shown in Figure (3). To choose an event at random, a random number between 0 and 1 is generated – for any range the random number falls in, the respective event occurs.

3 Results

3.1 Deterministic Model: Finding Equilibrium Points and Stability

By setting (3) equal to 0 it was determined that

$$x = 0, \tfrac{1}{2}(1 + k - y \pm \sqrt{1 - 2k + 2y + k^2 - 2ky + y^2 + 4bk}).$$

However, due to the difficulty of solving the cubic $\frac{dy}{dt}$ for y only 3 equilibria points were found analytically: $(0,0)$, $(0,1)$, $(0,k)$. Numerical integration of (3) and (4) for various parameter values showed that other equilibria existed. Alternate methods and the cases $b = 0$ and $q = 0$ were used to approximate these equilibrium points. We evaluated the system for $b = 0$ to observe how our model behaved if no antifungal agent was used. We then evaluated the system at $q = 0$ because we knew q was a small number and assumed perturbations would lead us to find all possible equilibrium points.

3.1.1 The Trivial Equilibrium Points

The Jacobian of the system of equations (3) and (4) is

$$\begin{pmatrix} (1-2x)(1-\frac{x+y}{k}) - \frac{x(1-x)}{k} - b & -\frac{x(1-x)}{k} \\ -\frac{ry(1-y)}{k} + bq & r(1-2y)(1-\frac{x+y}{k}) - \frac{ry(1-y)}{k} \end{pmatrix}.$$

At the point $(0,0)$ the Jacobian becomes

$$\begin{pmatrix} 1-b & 0 \\ bq & r \end{pmatrix}.$$

In this case the eigenvalues are $1-b$ and r. The value r is equal to $\frac{r_2}{r_1}$ and is a positive growth rate. If $b < 1$, the first eigenvalue is positive and $(0,0)$ is unstable. If $b > 1$, then this eigenvalue is negative and $(0,0)$ is a saddle point.

At the point $(0,1)$ the Jacobian becomes

$$\begin{pmatrix} \frac{k-1}{k} - b & 0 \\ bq & -r(\frac{k-1}{k}) \end{pmatrix}.$$

Here the eigenvalues are $-r\frac{k-1}{k}$ and $\frac{k-1}{k} - b$. The first eigenvalue $-r\frac{k-1}{k}$ will always be negative. Now consider the other eigenvalue. Because $k > 2$, $\frac{k-1}{k}$ will be positive. If $\frac{k-1}{k} > b$ then this eigenvalue will be positive, meaning $(0,1)$ is a saddle point. If $\frac{k-1}{k} < b$ then this eigenvalue will be negative implying that $(0,1)$ will be stable under this condition for b.

At the point $(0,k)$ the Jacobian is given by

$$\begin{pmatrix} -b & 0 \\ r(k-1) + bq & r(k-1) \end{pmatrix}.$$

This time the eigenvalues are $-b$ and $r(k-1)$. Note $-b$ is a negative eigenvalue and $r(k-1)$ is always positive. Thus, this equilibrium point is always a saddle point.

3.1.2 The Existence of a Non-Trivial Equilibrium Point

Based on numerical integration of (3) and (4), it appears that a stable interior equilibrium point lies within the region Ω, where $\Omega = \{(x,y) : 0 < x, 0 < y; x+y < k\}$. We therefore assume that, for equilibrium point (x_0, y_0),

$x_0+y_0 < k$. Because $\frac{dx}{dt} = 0$ and $\frac{dy}{dt} = 0$ at (x_0, y_0), we can analyze conditions for x_0 and y_0. Analyzing (3) and (4) the following was obtained:

$$x_0(1-x_0)(1-\frac{x_0+y_0}{k}) = bx_0$$
$$ry_0(1-y_0)(1-\frac{x_0+y_0}{k}) = -bqx_0$$

Noting signs, we see that $1-\frac{x_0+y_0}{k}$ is positive. Therefore x_0 must be less than one and greater than zero, and y_0 must be greater than one. This indicates that the interior equilibrium point must lie outside of the square bounded by $(1,1)$ and the origin.

Using a numerical solver for the values $q = 0.015$, $r = 0.4$, and $k = 3$, we plotted the values for x_0 and y_0 as functions of b in Figure (4).

As shown in the figure, this equilibrium point moves to the left with increasing b until it is out of the first quadrant, crossing through the equilibrium point at $(0,1)$. This occurs at approximately $\frac{2}{3}$. We will later show that more generally speaking this occurs approximately at $\frac{k-1}{k}$.

Further numerical analysis yields the existence of three other equilibria with varying positions. They will be discussed further in Section 4.1.5.

3.1.3 Stability Analysis of Non-trivial Equilibrium points

In order to analyze stability let $x_0 + y_0 = m$ where (x_0, y_0) is the non-trivial equilibrium from (3) and (4), and assume $0 < m < k$. At the moment assume (x_0, y_0) is an equilibrium with $x_0 > 0$ and $y_0 > 0$. We will also assume that $x_0 + y_0 < k$ or $k - m > 0$. If we multiply both sides by k, and set our new $\dot{x}$ equal to zero we have the equation,

$$(1-x_0)(k-m) - kb = 0$$
$$x_0 = 1 - \frac{kb}{k-m}. \tag{5}$$

Now if we set equation (4) equal to zero, and solve for y_0 we get,

$$y_0 = \frac{1}{2}\left(1 + \sqrt{1 + \frac{4bkqx_0}{r(k-m)}}\right). \tag{6}$$

The Jacobian of equations (3) and (4) is:

$$\begin{pmatrix} (1-\frac{x+y}{k})(1-2x)-\frac{1}{k}x(1-x)-b & -\frac{1}{k}x(1-x) \\ -\frac{r}{k}y(1-y)+bq & -ry(1-\frac{x+y}{k})-\frac{r}{k}y(1-y) \end{pmatrix}.$$

Using equations (5) and (6) we find that the Jacobian at (x_0, y_0) is:

$$x_0 b \begin{pmatrix} -\frac{1}{1-x_0}-\frac{1}{k-m} & -\frac{1}{k-m} \\ \frac{q}{k-m}+\frac{q}{x_0} & -\frac{q}{y_0}+\frac{q}{1-y_0}+\frac{q}{k-m} \end{pmatrix}.$$

We see that the trace(x_0, y_0)= $-\frac{1}{1-x_0}+\frac{q-1}{k-m}-\frac{q}{y_0}+\frac{q}{1-y_0}$. It is easy to see from equations (5) and (6) that $1-x_0>0$ and $1-y_0<0$. But also, because q is a probability, $q-1<0$ so that every term of the trace(x_0, y_0) is negative; therefore the trace of any non-trivial equilibrium is always negative.

Next we will look at the determinant. Algebraic simplification gives us

$$\text{detJ}(x_0, y_0) = (x_0 b)^2 q[(-\tfrac{1}{1-x_0}-\tfrac{1}{k-m})(-\tfrac{1}{y_0}+\tfrac{1}{1-y_0})-\tfrac{1}{(1-x_0)(k-m)}+\tfrac{1}{x_0(k-m)}]$$

The first quantities are both negative (since $1-y_0<0$), so their product is positive. The sum of the last two terms is $\frac{1-2x_0}{x_0(1-x_0)(k-m)}$.
One can see, from equation (5), that in the case $b>\frac{1}{2}$, $1-2x_0>0$. If we know that

$$1-x_0-x_0 = 2\frac{kb}{k-m}-1.$$

then the condition for the numerator to be positive is $2\frac{kb}{k-m}>1$, making the determinant positive. Given these conditions, the equilibrium point (x_0, y_0) is stable.

3.1.4 Case #1: $b=0$

Solving for $\frac{dx}{dt}=0$ and $\frac{dy}{dt}=0$ six possible equilibria were discovered when the anti-fungal agent is absent ($b=0$). The revised equations under this condition are

$$\frac{dx}{dt} = x(1-x)\left(1-\frac{x+y}{k}\right) \tag{7}$$

$$\frac{dy}{dt} = ry(1-y)\left(1-\frac{x+y}{k}\right). \tag{8}$$

There are five trivial equilibria, $(0,0)$, $(0,1)$, $(0,k)$, $(1,0)$, $(k,0)$, and one non-trivial equilibrium $(1,1)$. We also note that there is a line of equilibria, $x+y=k$, that both $(0,k)$ and $(k,0)$ lie on. We will first discuss the stability of the trivial equilibria.

The Jacobian of system (7-8) is now given by

$$\begin{pmatrix} (1-2x)(1-\frac{x+y}{k}) - \frac{x(1-x)}{k} & \frac{x(1-x)}{k} \\ -\frac{ry(1-y)}{k} & r(1-2y)(1-\frac{x+y}{k}) - \frac{ry(1-y)}{k} \end{pmatrix}.$$

At the point $(0,0)$ the Jacobian becomes

$$\begin{pmatrix} 1 & 0 \\ 0 & r \end{pmatrix}.$$

Both 1 and r are positive eigenvalues, so the point $(0,0)$ is always unstable.

At the point $(0,1)$ the Jacobian becomes

$$\begin{pmatrix} 1-\frac{1}{k} & 0 \\ 0 & -r(1-\frac{1}{k}) \end{pmatrix}.$$

Because we assume $k > 2$, $1-\frac{1}{k}$ is a positive term. It also follows that $-r(1-\frac{1}{k})$ is a negative term. Therefore the point $(0,1)$ is always a saddle point.

The Jacobian for the point $(1,0)$ is

$$\begin{pmatrix} -(1-\frac{1}{k}) & \frac{1}{k} \\ 0 & r(1-\frac{1}{k}) \end{pmatrix}.$$

Our first eigenvalue, $-(1-\frac{1}{k})$, is negative, while the second, $r(1-\frac{1}{k})$, is positive, so the point $(1,0)$ is always a saddle point.

At our non-trivial equilibrium point $(1,1)$ the Jacobian is

$$\begin{pmatrix} -(1-\frac{2}{k}) & 0 \\ 0 & -r(1-\frac{2}{k}) \end{pmatrix}.$$

Because we assume $k > 2$, both eigenvalues, $-(1-\frac{2}{k})$ and $r(1-\frac{2}{k})$, are negative, and our non-trivial equilibrium is stable.

The Jacobian at the points $(0,k)$ and $(k,0)$, or any point on the line $x+y=k$, results in a row of zeros, giving us zero eigenvalues. Initially we

considered using higher order linearizations, but after inspecting the eigenvectors our zero eigenvalues made more sense, and higher order linearizations would have proven futile: taking the Jacobian of our original equations (3) and (4), about the point $(0, k)$ we get the following eigenvectors and eigenvalues:

$$\lambda_1 = -b$$
$$v_1 = \left(1, -1 + \frac{b(1-q)}{b + r(k-1)}\right),$$

and

$$\lambda_2 = r(k-1)$$
$$v_2 = (0, 1).$$

The first eigenvector points in the direction of the line $x + y = k$ when $b = 0$, but the eigenvalue is also zero. This is because the line $x + y = k$ is a line of unstable equilibrium points. The eigenvalue is zero because no point on the line $x + y = k$ is any more attractive than any other (see Figure (5)).

3.1.5 Case #2: $q = 0$

In this section the rescaled model is

$$\frac{dx}{dt} = x(1-x)\left(1 - \frac{x+y}{k}\right) - bx \tag{9}$$
$$\frac{dy}{dt} = ry(1-y)\left(1 - \frac{x+y}{k}\right). \tag{10}$$

Here we consider the possibility that the anti-fungal agent is effective in killing the susceptible strain, $b > 0$, and the dosage is taken properly and that there is no mutation of x into y, $q = 0$.

Our equilibrium points are $(0,0)$, $(0,1)$, $(0,k)$, $(\frac{1}{2}(k+1) \pm \sqrt{(k-1)^2 + 4bk}, 0)$, and $(\frac{1}{2}k \pm \frac{1}{2}\sqrt{(k-2)^2 + 4bk}, 1)$. The Jacobian for system (9-10) is

$$\begin{pmatrix} (1-2x)(1-\frac{x+y}{k}) - \frac{x(1-x)}{k} - b & -\frac{x(1-x)}{k} \\ -\frac{ry(1-y)}{k} & r(1-2y)(1-\frac{x+y}{k}) - \frac{ry(1-y)}{k} \end{pmatrix}.$$

We have already evaluated the stability of the points $(0,0)$, $(0,1)$, and $(0,k)$ in Section 4.1.1. Therefore we will concern ourselves with the remaining four.

The Jacobian for the point $(\frac{1}{2}(1+k+\sqrt{(k-1)^2+4bk},0)$ is

$$\begin{pmatrix} \frac{1}{2}\frac{(k-1)^2+4bk+(k+1)\sqrt{(k-1)^2+4bk}}{k} & \frac{1}{4}\frac{(1+k+\sqrt{(k-1)^2+4bk})(-1+k+\sqrt{(k-1)^2+4bk})}{k} \\ 0 & -\frac{1}{2}\frac{r(-k+1+\sqrt{(k-1)^2+4bk})}{k} \end{pmatrix}.$$

Our first eigenvalue is $\frac{1}{2k}((k-1)^2+4bk+(k+1)\sqrt{(k-1)^2+4bk})$, and is clearly always positive because all of its terms are positive. Since $1-k<0$ and $b>0$ the second eigenvalue, $-\frac{1}{2k}(r(-k+1+\sqrt{k^2+4bk-2k+1}))$, is positive. Thus the point $(\frac{1}{2}(1+k+\sqrt{(k-1)^2+4bk},0)$ is always unstable.

At the point $(\frac{1}{2}(1+k-\sqrt{(k-1)^2+4bk},0)$ the Jacobian is

$$\begin{pmatrix} \frac{1}{2}\frac{(k-1)^2+4bk-(k+1)\sqrt{(k-1)^2+4bk}}{k} & \frac{1}{4}\frac{(-(k+1)+\sqrt{(k-1)^2+4bk})(1-k+\sqrt{(k-1)^2+4bk})}{k} \\ 0 & \frac{1}{2}\frac{r(k-1+\sqrt{(k-1)^2+4bk})}{k} \end{pmatrix}.$$

Our new eigenvalues are $\frac{1}{2k}((k-1)^2+4bk-(k+1)\sqrt{(k-1)^2+4bk})$ and $\frac{1}{2k}(r(k-1+\sqrt{(k-1)^2+4bk}))$. We know that the latter is always positive because all of the terms are positive. Also $k=\frac{k_2}{k_1}>1$ implies $k-1>0$, r is a positive ratio between r_2 and r_1, and we have already shown that $(k-1)^2+4bk$ is always positive. The former eigenvalue is dependent on b. If $b>1$ then this eigenvalue is positive, and $(\frac{1}{2}+\frac{k}{2}-\frac{1}{2}\sqrt{(k-1)^2+4bk},0)$ is unstable. However, if $b>1$ the equilibrium point is in the second quadrant. Therefore, this equilibrium can be considered nonexistent in a biological sense. Inversely, if $b<1$ then the point is in the first quadrant and this eigenvalue is negative. Thus this point is a saddle point.

The Jacobian for the point $(\frac{k}{2}+\frac{1}{2}\sqrt{(k-2)^2+4bk},1)$ is

$$\begin{pmatrix} \frac{1}{2}\frac{(k-2)^2+4bk+k\sqrt{(k-2)^2+4bk}}{k} & \frac{1}{4}\frac{(k+\sqrt{(k-2)^2+4bk})(k-2+\sqrt{(k-2)^2+4bk})}{k} \\ 0 & \frac{1}{2}\frac{r(2-k+\sqrt{(k-2)^2+4bk})}{k} \end{pmatrix}.$$

The eigenvalues are $\frac{1}{2k}((k-2)^2+4bk+k\sqrt{(k-2)^2+4bk})$ and $\frac{r}{2k}(2-k+\sqrt{(k-2)^2+4bk})$. The former is always positive. The latter is also always positive but could use a bit more explanation. The sign of our eigenvalue depends on $-k+2+\sqrt{(k-2)^2+4bk}$. Because $b>0$, $|-k+2|<\sqrt{(k-2)^2+4bk}$. So this eigenvalue is always positive, and the point $(\frac{k}{2}+\frac{1}{2}\sqrt{(k-2)^2+4bk},1)$ is unstable.

At the point $(\frac{k}{2} - \frac{1}{2}\sqrt{(k-2)^2+4bk}, 1)$ our Jacobian becomes

$$\begin{pmatrix} \frac{1}{2}\frac{(k-2)^2+4bk-k\sqrt{(k-2)^2+4bk}}{k} & \frac{1}{4}\frac{(-k+\sqrt{(k-2)^2+4bk})(2-k+\sqrt{(k-2)^2+4bk})}{k} \\ 0 & -\frac{1}{2}\frac{r(k-2+\sqrt{(k-2)^2+4bk})}{k} \end{pmatrix}.$$

The eigenvalues are $\frac{1}{2k}((k-2)^2+4bk-k\sqrt{(k-2)^2+4bk})$ and $-\frac{1}{2k}(r(k-2+\sqrt{(k-2)^2+4bk}))$. $b > 0$ implies that $k-2 < \sqrt{(k-2)^2+4bk}$. So the second eigenvalue is negative. As long as $b > \frac{k-1}{k}$, the first eigenvalue is positive, and this equilibrium point is a saddle point. However, when $b > \frac{k-1}{k}$ the equilibrium point becomes negative – meaning that this equilibrium point is non-existent in a biological sense. When $0 < b < \frac{k-1}{k}$ the eigenvalue is negative, and this equilibrium is stable.

The following is a table summarizing stability and conditions under which the equilibria points are positive for different values of b.

Equilibria Points	b		
	$(0, \frac{k-1}{k})$	$(\frac{k-1}{k}, 1)$	$(1, \infty)$
$(0,0)$	U	U	$\pm$
$(0,1)$	$\pm$	S	S
$(0,k)$	$\pm$	$\pm$	$\pm$
$(\frac{1}{2}(1+k+\sqrt{(k-1)^2+4bk}), 0)$	$\pm$	$\pm$	$\pm$
$(\frac{1}{2}(1+k-\sqrt{(k-1)^2+4bk}), 0)$	$\pm$	$\pm$	DNE
$(\frac{k}{2}+\frac{1}{2}\sqrt{(k-2)^2+4bk}, 1)$	U	U	U
$(\frac{k}{2}-\frac{1}{2}\sqrt{(k-2)^2+4bk}, 1)$	S	DNE	DNE

Note: S =Stable, U =Unstable, DNE =Does not exist, $\pm$ =Saddle

3.1.6 Connecting the Case $q = 0$ to the Bifurcation graph (Figure (10))

In general our q value is very small. About 99% of all strains of *Candida albicans* are susceptible. So our results for the case $q = 0$ are very close to situations when q is very small. After examining the bifurcation graph at $q = 0.015$, the average value we found for q, we noticed that our results were similar. In fact, we numerically found that a perturbation about q for the $q = 0$ case would have proven accurate up to three decimal points.

In the $q = 0$ case we found that when $b = \frac{k-1}{k}$ the stable interior equilibrium point crashes into the saddle point located at $(0, 1)$, transferring

its stability (Figure (7)). For $b < \frac{k-1}{k}$ both points exist (Figure (6)). For $b > \frac{k-1}{k}$ the saddle point becomes negative, and unimportant to our system. This change corresponds to the leftmost curve in Figure (10).

At $b = 1$, which can be read directly from the bifurcation graph, the saddle point on the x-axis disappears. As b increases from zero to 1, the saddle point moves toward the unstable equilibrium at $(0, 0)$. When they collide $(0, 0)$ becomes a saddle point (Figure (8)). This is shown by the rightmost vertical line in the bifurcation graph and in our discussion of the local stability at the point $(0, 0)$.

We could not approximate the horizontal line on the bifurcation graph. We did find that the line corresponded to the appearance and disappearance of two equilibrium points in a saddle-node bifurcation, one unstable and one saddle point, that, if they existed, were born on the stable manifold of the $(0, k)$. This stable manifold forms the basin boundary, analogous to the unstable line $x + y = k$ in the $b = 0$ case (Figure (9)). We also noticed that if we extended our bifurcation graph into the second quadrant, the line we initially thought was exponential displayed erratic, distinctly non-exponential behavior. By trying different parameter values we were able to move the portions of the horizontal line that displayed unusual behavior into the first quadrant. At the values $r = 0.0001$, $b = 0.01$, $k = 6.8$, and $q = 0.015$ we actually found that there were two stable interior points, three saddle points, two along the axes and one between the stable points, and one unstable point at $(0, 0)$. Finding a situation where there was two stable points seemed very promising biologically, as it gave us more possible stable levels where the fungus could live in the body harmlessly, but the parameters are not biologically realistic. Our value $r = \frac{r_2}{r_1}$ is, by virtue of $r_2 < r_1$, between 1 and 0, but r_2 would have to be substantially smaller than r_1 for this to happen. Because we are dealing with the same species, but different strain of a fungus, their respective growth rates could not differ by a factor of 0.0001.

3.2 Stochastic Results

The stochastic simulation for the most part supports the deterministic results. The simulations show that for a sufficient value of b, x is killed off while leaving a resident population of y (Figure (11)). This value, which happens to be, approximately, $b > \frac{k-1}{k}$ only applies whenever the initial x population is less than some value corresponding to boundary of the deterministic basin of attraction. However, for values of $b < \frac{k-1}{k}$ a stable population of x and y

remain present (Figure (12)). Also for some values of b there appears to be an equal chance of either the x population growing exponentially or the x population going extinct and leaving a resident y population. Under the condition that the initial x population is greater than some value corresponding to boundary of the deterministic basin of attraction the population takes off rapidly (Figure (13)). In order to suppress this outbreak, it is necessary to drastically increase the value of b (Figure (14)).

4 Discussion

Results from the deterministic model indicate that there are treatment rates at which a stable population of resistant bacteria and non-resistant bacteria is established. Although our model does not show this, we can assume that at these instances the susceptible population remains resident and the resistant population goes to near extinction due to its smaller reproductive rate and competition with the susceptible population for resources. However, at higher treatment rates the susceptible population is driven to extinction leaving behind only a resident resistant population. This is significant because if a patient's immune system were to be compromised at this state, doctors would have a more difficult strain of *C. albicans* to treat. Results from the stochastic simulation provide support for these conclusions about variable treatment rates. Also from stochastic simulations it is clear that misuse of antifungal treatment can create harmful situations in which more resistant strains can develop. For example, the use of treatment before any harmful symptoms appear in hopes of destroying any resident non-resistant strains can result in the creation of more resilient strains. However, it appears that if used responsibly–meaning for the necessary period of time and in the correct dosage–the chance development of resistant strains can be minimized, as simulation results reflect.

Possible future studies include investigating the $k < 2$ case, if such a case biologically exists. Also if possible, we would like to determine strict parameters for r, to the exclusion or inclusion of the cases where there are two stable interior points. We would also like to expand our model to include multiple strains of *Candida albicans* of varying resistance. In addition, it would be interesting to see if competition at resident population could be included in the model. Finally, we would like to modify the model to take into account a death rate for the resistant populations, any possibility of backwards

mutation into a susceptible strain, and the immune systems strength.

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Baojun Song
Steven Tennenbaum

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A Matlab code for the Stochastic Simulations

```
function state =candida(SA,RA,b,q,r1,r2,k1,k2,tfinal,hm)
close
hold on
for i = 1:hm
t=0;
total_rate=1;
gen=0;
S=SA;
R=RA;
state=[t S R];
while (gen < tfinal) & total_rate>0
R_S = r1*S*(1-S/k1)*(1-(S+R)/k2);
R_B = b*S;
R_R = r2*R*(1-R/k1)*(1-(S+R)/k2);
A = abs(R_S);
B = abs(R_R);
total_rate = A+B+R_B;
if total_rate>0;
time_to_next = -log(rand)/total_rate;
t = t+time_to_next;
P=R_B/total_rate;
PR=B/total_rate;
PB=q*P;
r=rand;
if r<P
S=S-1;
if r<PB;
R=R+1;
end
end
if P<r & r<(PR+P)
if R_R>0
R=R+1;
else
R=R-1;
end
```

```
end
if r>PR+P & r<1
R_S>0
S=S+1;
else
S=S-1;
end
end
if S<0
S=0;
end
curr_state=[t S R];
state=[state;curr_state];
gen=gen+1;
end
end
x=state(:,1);
y1=state(:,2);
y2=state(:,3);
plot(x,y1,'b-',x,y2,'r-');
end
legend('Non-resistant','Resistant')
```

B Figures

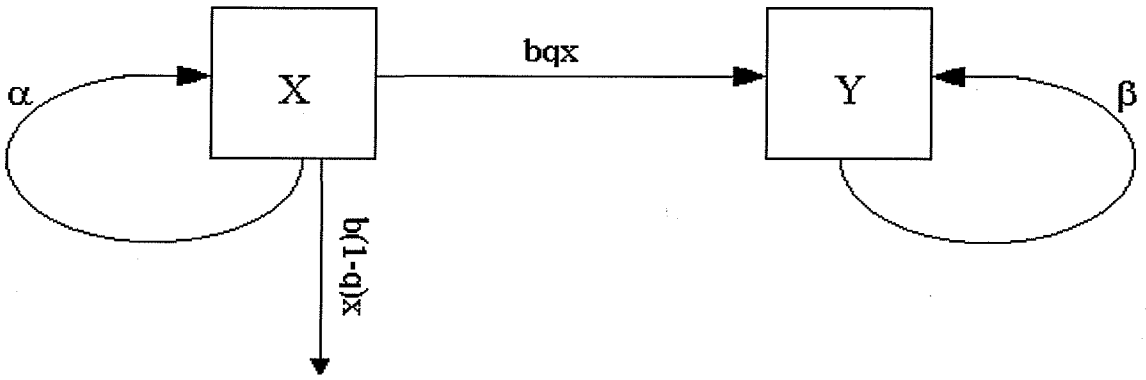

Figure 1: Graphical representation of the deterministic model: $\alpha = r_1 x(1 - \frac{x}{k_1})(1 - \frac{x+y}{k_2})$ and $\beta = r_2 y(1 - \frac{x}{k_1})(1 - \frac{x+y}{k_2})$

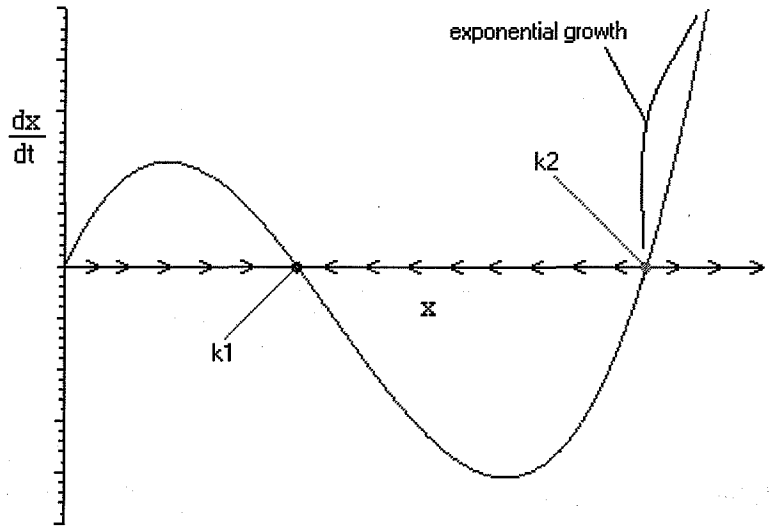

Figure 2: Graph of $\dot{x}$ vs. x for b =0

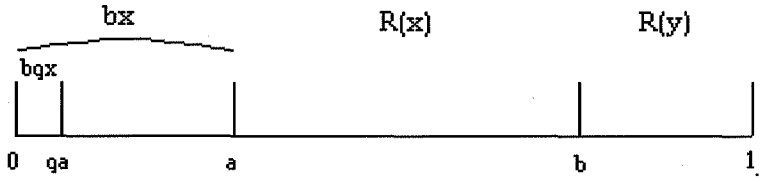

Figure 3: A probability distribution for choosing the next event

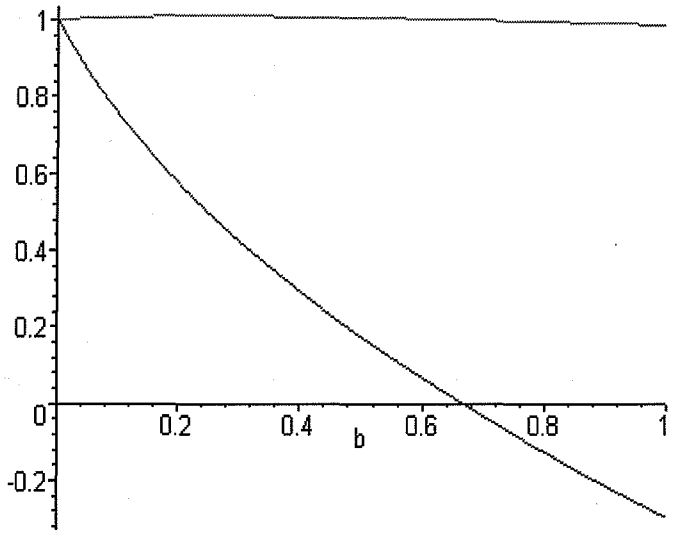

Figure 4: The top line is a plot of y_0 as function of b. The bottom line is a plot of x_0 as a function of b.

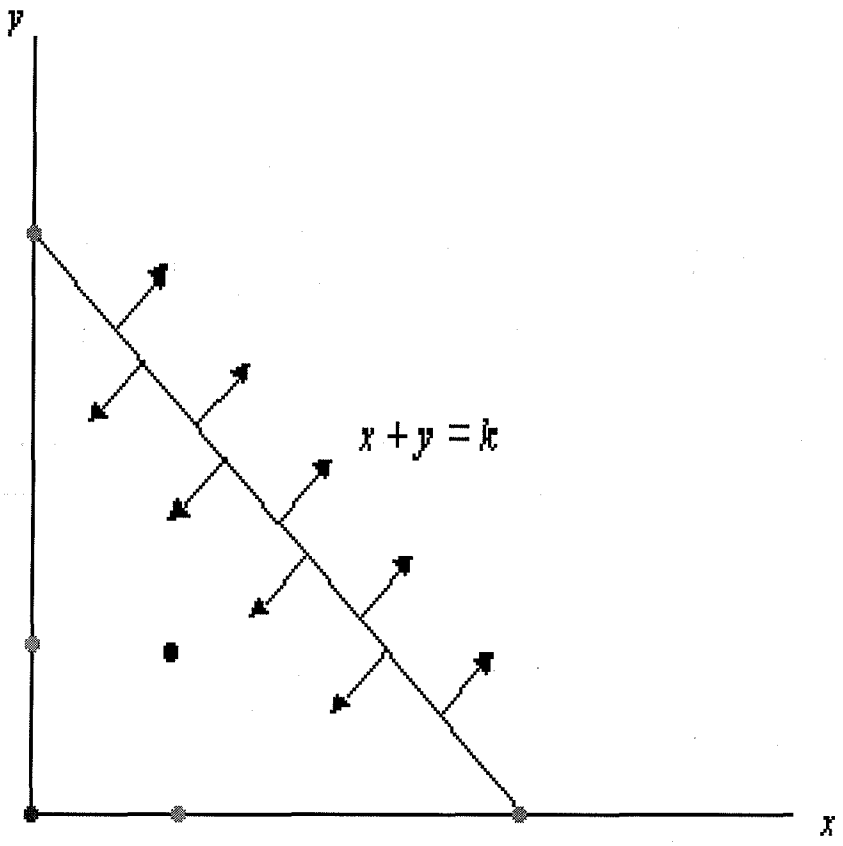

Figure 5: Phase-plane diagram for when $b = 0$. $x + y = k$ represents a line of unstable points. Trajectories within the region are drawn to the stable equilibrium point. Trajectories outside the region go towards infinity.

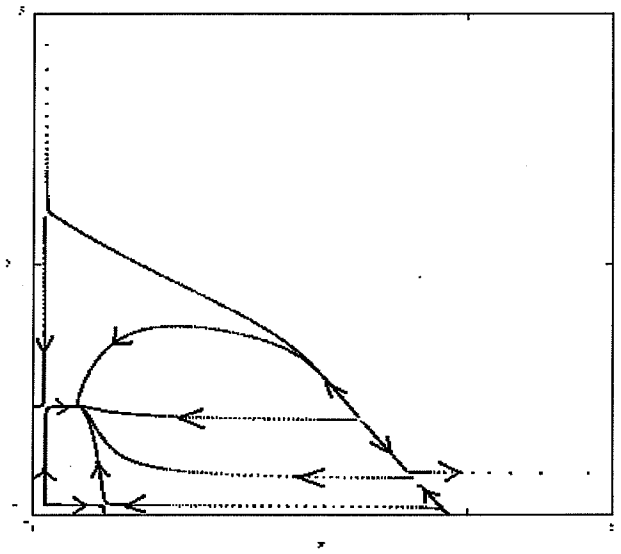

Figure 6: Seven equilibrium points: Non-trivial equilibrium point still exists. [14]

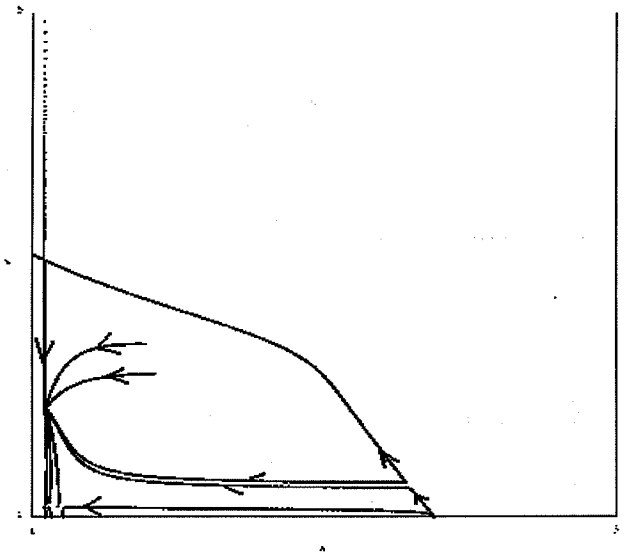

Figure 7: Six equilibrium points: After stable interior equilibrium point crashes into saddle at (0,1) [14]

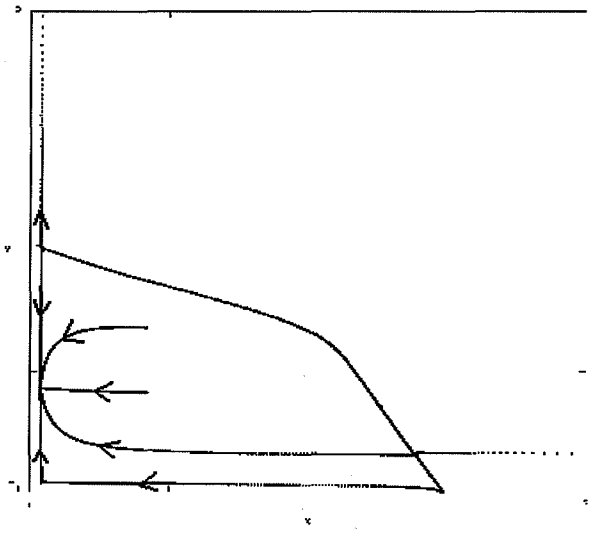

Figure 8: Five equilibrium points: After the saddle crashes into the unstable point at (0,0) [14]

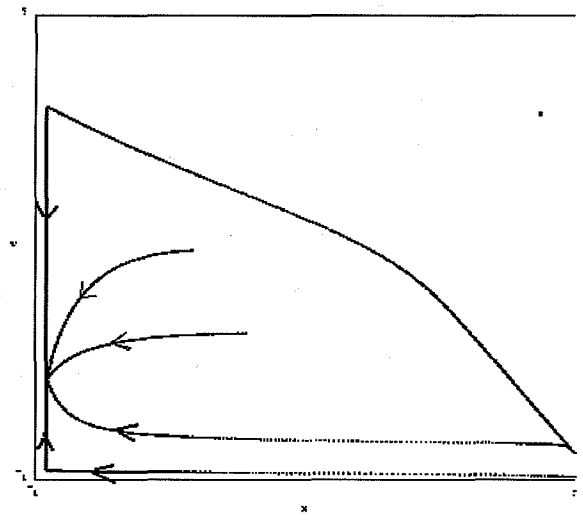

Figure 9: Three equilibrium points: After the saddle and unstable equilibria lying along the boundary annihilate each other. [14]

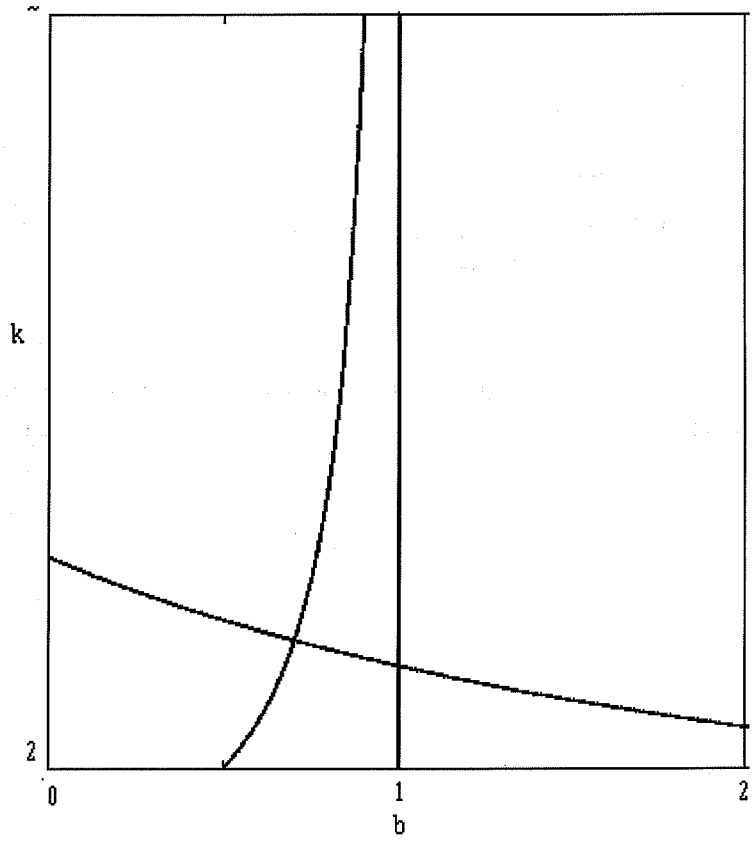

Figure 10: Bifurcation graph of deterministic model for $r = 0.4$ and $q = 0.015$ [14]

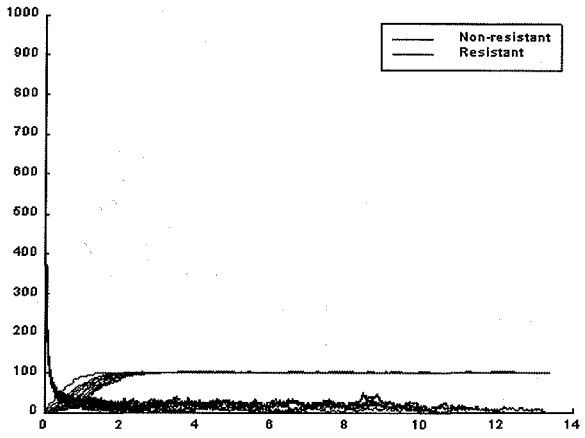

Figure 11: parameter values=[b=7, q=0.015, r1=10, r2=3, k1=100, k2=1000, x0=1000] The non-resistant x population is going extinct and is being replaced by a resident resistant y population

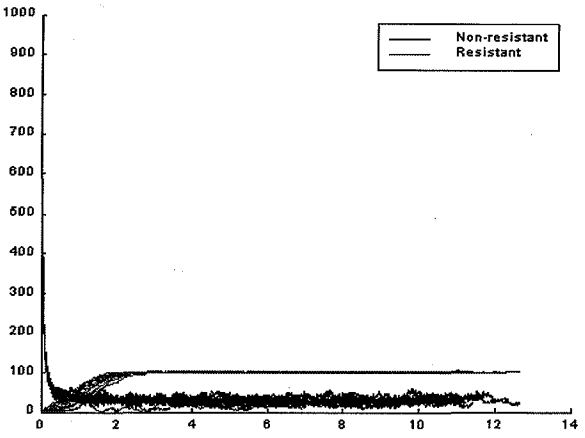

Figure 12: parameter values=[b=6, q=0.015, r1=10, r2=3, k1=100, k2=1000, x0=1000] The non-resistant x population nearly goes extinct but a resistant y population is also established

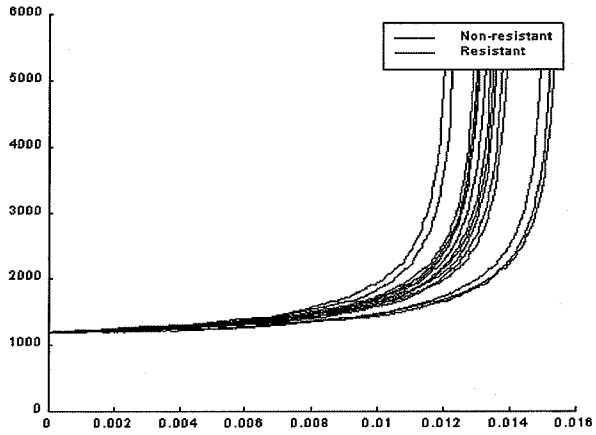

Figure 13: parameter values=[b=11, q=0.015, r1=10, r2=3, k1=100, k2=1000, x0=1200] The initial non-resistant x population is increased past k_2 (equal to 1000) by 200; the non-resistant population explodes.

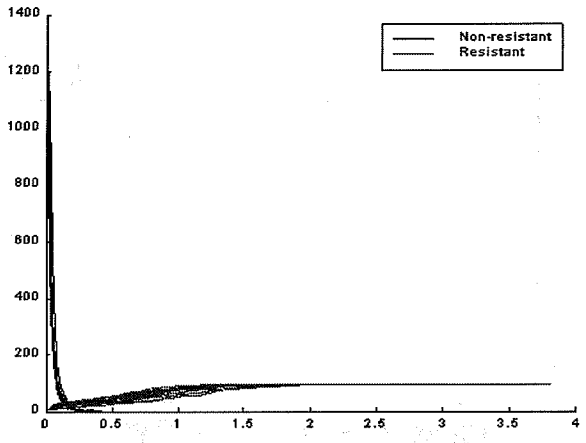

Figure 14: parameter values=[b=25, q=0.015, r1=10, r2=3, k1=100, k2=1000, x0=1200] b (treatment rate) which was 11, is increased to 25, the point at which the explosion is suppressed and the non-resistant x population is driven to extinction and a resistant y population develops.