

An Algorithm for Optimizing Rotational Control of *Globodera rostochiensis* on Potato Crops in Bolivia

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Abstract: The outline area of new cysts of *Globodera rostochiensis* was measured by image analysis. A linear regression of this value against egg content provided a basis for adjusting the egg number for cyst size. This adjusted egg content provides an estimate of the relative fullness of a cyst with eggs. This value showed an exponential decline in eggs over 3.5 years since the last potato crop. It corresponds to an average loss in the dormant egg population of $32.8 \pm 5.6\%$ /year for 26 fields at Toralapa, Bolivia. This value compared well with a mean decline of $40 \pm 4\%$ /year for 42 fields after measuring viable eggs/100 g soil on two occasions one year apart. The new approach allows declines to be estimated at one time point. The decline in lipid content of the dormant, unhatched second-stage juveniles (J2) was $17 \pm 6\%$ per annum as measured by image analysis after Oil red O staining. This may be sufficient to compromise infectivity after 3 to 4 years of dormancy. A standard model was modified to consider the effect of both lipid depletion during dormancy and choice of susceptible potato on the population dynamics of *G. rostochiensis* under rotational control. It is concluded that a few cultivars may impose lower populations on *G. rostochiensis* in 3 to 4-year rotations than the majority used in Bolivia.

Key words: Bolivia, decline rate, dormancy, fecundity, *Globodera rostochiensis*, lipid use, nematode, pest management, population models, potato, potato cyst nematode, rotation.

Our study of the physiological ecology of *Globodera rostochiensis* in Bolivian fields has shown a seasonal variation in readiness of second-stage juveniles (J2) to hatch (Holz et al., 1998). The fecundity of this nematode at one locality varied by ca. 15-fold on a range of susceptible cultivars used by growers in Bolivia (Holz et al., 1999). The initial lipid reserves available to J2 were also influenced by the cultivar on which its parental female developed. Lipid reserves subsequently decline slowly for J2 of *G. pallida* during dormancy in United Kingdom soils. The extent of lipid reserves when dormancy ends influences the subsequent infective life of the J2 of *Globodera* spp. Lipid is utilized rapidly after hatching (Robinson et al., 1985; Storey, 1984).

The initial population (P_i) of *G. rostochiensis* at potato planting influences yield loss (McSorley and Phillips, 1993). A value of 10 viable eggs/g soil is often given as the economic threshold. The relationship between P_i and the population after reproduction (P_f) has been modeled (Phillips et al., 1991). A single generation of the nematode normally occurs on potato in the valles and Alti Plano of Bolivia. Population increase on potato is countered in rotational control by an annual decline in the dormant egg population when potato is not cropped. This decline is normally considered to be exponential (Jones and Kempton, 1978). It can be enhanced experimentally in Bolivia by use of certain lines

of two traditional nonhost crops, lupin and quinoa, in rotations (Franco et al., 2000), but current rotational control of 3 to 4 years in the valles region of Bolivia is often inadequate. *Globodera* spp. occur in about 45% of potato fields in Cochabamba Department of Bolivia, and damaging populations at planting of >35 viable eggs/g soil are very common (Franco et al., 1999). We now report both population decline and lipid utilization rates for dormant populations in Bolivia. This and our earlier data (Holz et al., 1999) are used to enhance a population model. Our aim is to determine if potato can be grown in Bolivia using a 3 or 4-year rotation with limited yield losses due to *Globodera* spp.

MATERIALS AND METHODS

Measurements of *G. rostochiensis* density in soil and eggs/cyst: Cysts were collected from soil from fields at the field station of Programa de Investigación de la Papa (PROINPA), Toralapa, and from fields on the Alti Plano. The population at Toralapa is *G. rostochiensis* (Holz et al., 1999). Cysts were extracted using a Fenwick can (Shepherd, 1970). Viable eggs per gram soil were measured for 100-g soil samples of the populations from the Alti Plano by standard procedures (Shepherd, 1970). Values were obtained for 42 field samples of crop-free land before fallow or planting of one of four non-host crops (barley, broad bean, quinoa, or tarwi). They were measured again 1 year later just prior to cropping with potato. The outline area of cysts collected from 23 fields at Toralapa was measured using image analysis (see below). The egg content of at least 25 cysts per field was obtained by opening individual cysts with needles under a stereo binocular microscope and counting the eggs that each contained. The overall decline rate for 42 non-host fields on the Alti Plano was analyzed using multiple ANOVA and a standard statistical package program (SPSS 9.0, SPSS Inc., Chicago, IL) run on a personal computer.

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Image analysis and lipid measurements: Cyst outline area was measured under a compound microscope (Leitz DMRB) fitted with a color camera (Kappa CF 15 MCC), which was connected to a frame grabber board. Analysis was controlled with Quantimet 500 software (Leica, London, UK). Dimensions were calibrated using a micrometer slide. Lipid levels were measured by image analysis after Oil red O staining of J2 using previously described procedures (Holz et al., 1999).

Development of population model: The work was centered on a standard population model (Phillips et al., 1991). The model can be stated as:

$$P_f = \frac{f' (1 - C_p) P_i}{1 + b' (1 - C_p) P_i (1 + P_i/c)} + C_p P_i$$

The variables and their values used in this work are as follows. P_f = population of viable eggs per gram of soil at harvest and so is calculated by the model. P_i = population of viable eggs per gram of soil at planting and is set at 10 for first iteration of the model. $C_p = 0.2$ is a typical value for the proportion of viable eggs not hatching when potatoes are grown in the highlands of Bolivia. f' = a fecundity and establishment value. It was set at $\times 45$ and multiplied by a fecundity value if the latter is >1 . $b' = 0.3$ is the proportion of animals becoming males. $e' = 0$ to 0.9 is the loss of fecundity relative to the mean for 23 cultivars (Holz et al., 1999). The value for f' of 45 was chosen as it provided a P_f of 10 after 5 generations when both relative fecundity and relative lipid are set as 1.0 and there are 6 non-host years per rotation cycle. Table 1 summarizes the modified inputs provided to the model. It was developed within a spreadsheet to generate the values provided in the results.

RESULTS

Annual decline of G. rostochiensis in non-host years at Toralapa: The analysis established that the four non-host crops and fallow all gave similar decline rates. The pooled value was $40 \pm 4\%$ /year (mean \pm standard error of the mean).

The outline area was measured for more than 450 newly formed cysts from 12 fields at Toralapa before each cyst was opened, and the eggs it contained counted under a binocular microscope. The data provided an overall linear regression of eggs per cyst against cyst outline area. The regression equation was then used to adjust the egg content of other cysts for differences in their size. This adjustment was made after measuring cyst size and egg number of at least 25 cysts collected from each of 26 fields at 0.5 to 3.5 years after the last potato crop. The fall in adjusted eggs per cyst with time since potato harvest provided an estimate of annual exponential decline of $32.8 \pm 5.6\%$ /year (regression coefficient \pm its standard error) (Fig. 1).

Enhancements to the population dynamics model: The standard population model can be enhanced if additional factors are considered. The initial lipid reserves of an unhatched dormant *G. rostochiensis* J2 are utilized during its dormancy in the egg. This decline in lipid reserves was plotted for the population at Toralapa (Fig. 2). The exponential curve represents an annual loss in lipid content of $17 \pm 6\%$ per year (regression coefficient \pm its standard error).

The initial lipid reserves of J2 also vary with the cultivar on which the parental female develops (Holz et al., 1999). The value for relative initial lipid has been multiplied by the proportion of reserves remaining after their exponential decrease during dormancy. This

TABLE 1. The values used to modify the standard population model.

Variable	Name	Formula	Comment
Number of non-host years in rotation	N		3 in a 4-year rotation
Lipid use in years of dormancy	L	$L = 1 * e^{-0.171 * Y}$	Y is number of years since the juvenile was formed years since last potato crop
Relative initial lipid I	I		Values of 0.63–1.32-fold defined for 23 cultivars by Holz et al. (1999)
Modified P_i Number of rotation cycles	$P_{im} = P_i I L$ C	P_i iterated for next rotation from previous P_f for C times using standard model	Numbers of rotation courses until P_i stabilizes; maximum value for C is 5
Overall survival of PCN eggs between potato crops	ΣS	$\Sigma S = 1 - D_1 - D_2 - \dots - D_n$	D_n is decline in n^{th} year of the rotation. Taken as 0.328/year (see Results) Declines P_{fc} to value P_{ic-1}

The Standard population model is from Phillips et al., 1991.

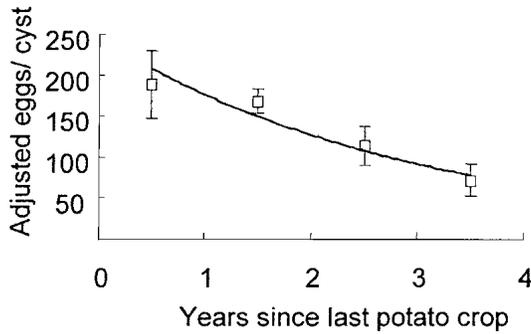


FIG. 1. Estimated adjusted egg content for cysts equalized for size against time since the last crop. The adjustment was made using a linear regression equation relating egg number of new cysts to their size (see text). The exponential curve suggests a decline of $32.8 \pm 5.6\%$ /year.

provides a modified value of effective P_i . The latter value defines the density of parasites likely to infect the potato plants. We assume that the probability of infection by a hatched J2 is linearly correlated with the lipid level available for it to locate a root and establish in it as a feeding parasite. The cultivar of potato is the final factor of importance we have considered. The fecundity of females varied 15-fold according to the susceptible cultivar parasitized (Holz et al., 1999). This effect on fecundity has also been included by altering f' and e' (see Materials and Methods) in the standard model (Phillips et al., 1991).

One application of the enhanced model is to determine if variation in initial lipid level of J2 has a measurable effect on population dynamics. This is obtained as the proportion of dormant eggs that must survive to maintain an effective P_i of 10 viable eggs/g soil after several rotation courses when females show varying levels of fecundity (Fig. 3). It indicates that the range of lipid levels reported by Holz et al. (1999) of 0.63 to 1.32-fold might alter the minimum rotation periods possible to avoid potato yield loss by a year.

A second application of the model is to explore the effect of different relative levels of female fecundity due to cultivar parasitized. The range is ca. 15-fold spanning $0.11\times$ to $1.63\times$ relative to the overall mean fecundity for

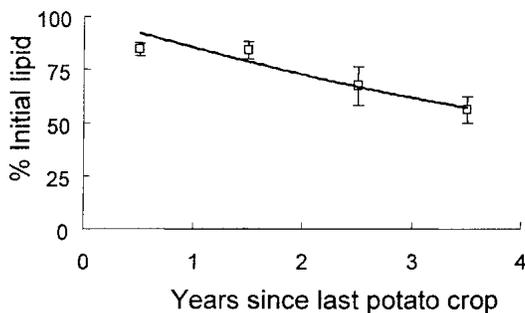


FIG. 2. Relative lipid content of juveniles with duration of dormancy under field conditions in Bolivia. Lipid levels were expressed as a percentage of values for newly formed J2. They are plotted against years since the last potato crop in that soil.

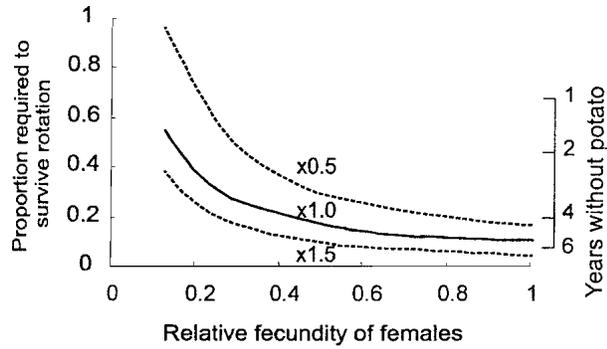


FIG. 3. Proportion of eggs that must survive to provide an initial density at potato planting P_i of $c10$ viable eggs/g soil for different relative fecundity of females and the initial lipid levels of their J2 progeny. The relative lipid values are 1.5-fold, 1-fold, and 0.5-fold of the mean levels found for all cultivars. The proportion of eggs required to survive is converted to number of years without potato cropping that would result in that value. This assumes the annual population decline is 32.8% per year. The range of initial lipid levels reported by Holz et al. (1999) was 0.65 to 1.32-fold.

females developing for all the susceptible cultivars examined by Holz et al. (1999). This provides one axis in Figure 4. A second axis represents compounded declines for years without potatoes. The figure shows predicted P_i values after several courses of a rotational control scheme when different compounded egg decline rates occur and cultivars allowing different fecundities are grown.

DISCUSSION

The high correlation between cyst size and egg content for newly formed cysts has been reported before for *Globodera* (Urwin et al., 1997). The outline area of a cyst is therefore a reliable indicator of the number of eggs it contains when newly formed, and the adjusted egg content for cysts that are not newly formed provides an estimate of egg loss. Therefore, loss of eggs can be estimated on an annual basis if cyst age and size are known. Multiplication of cysts is normally sufficient to ensure that those carried over from previous generations will not distort the relationship except at very high P_i values. The estimate of population decline using this

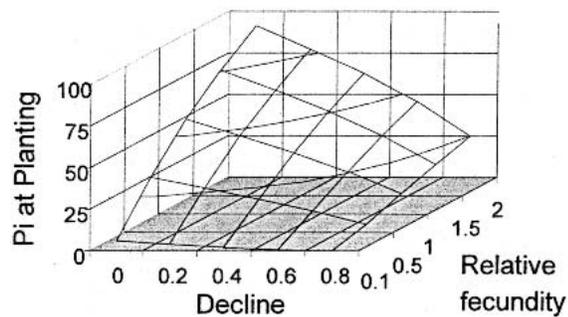


FIG. 4. The predicted long-term P_i established for different declines between potato crops and for cultivars that support different fecundities of *G. rostochiensis*. The relative lipid of new eggs is set at 1.

TABLE 2. Comparison of the long-term P_i likely for five cultivars of potato grown in Bolivia based on the relative fecundities and initial egg lipid levels.

Cultivar	Relative fecundity of females	Relative lipid	Predicted viable eggs/g soil at planting	
			3-year Rotation compounded decline = 0.59	4-year Rotation compounded decline = 0.74
Gendarme	0.11	0.93	3	1
Maria Huanca	0.18	0.84	6	2
Waycha	0.91	1.32	75	67
Imilla Blanca	1.05	1.21	78	71
Kosi	1.63	1.12	104	98

The damage threshold for P_i is ca. 10 viable eggs/g soil. Values are set to provide P_f of 10 eggs/g with a 7-year rotation.

approach was $32.8 \pm 5.6\%$ /year at Toralapa. The value is similar to that obtained for fields in the Alti Plano by standard methods. It is also similar to typical decline rates for this species in the very different environment of United Kingdom agriculture (Whitehead and Turner, 1998). The new approach may allow decline rates to be estimated from measurements on a single occasion, providing lapsed time since the last potato crop is known. Most farmers can recall that information. The approach may not be useful for those cyst nematodes such as *Heterodera glycines* that retain a variable proportion of their eggs in the cyst.

The dormant, unhatched J2 population of *G. rostochiensis* at Toralapa showed an exponential decline in lipid reserves of $17 \pm 6\%$ year. This rate compounds to a 50% loss of lipid after 4 years dormancy in the field. This is sufficient to suggest that prolonged dormancy suppresses infectivity. In contrast, dormant J2 *G. pallida* in Yorkshire, United Kingdom, took more than 7.5 years to use 50% of their reserves (Storey, 1984). Lipid utilization varies with *Globodera* spp. (Robinson et al., 1987a, 1987b), and further work may establish that population, locality, and the duration of the prevailing rotation also influence rates of lipid use. The enhanced population model assumes a linear relationship between the lipid level of newly hatched J2 and the probability of it establishing as a parasite under field conditions. A correlation between loss of lipid reserves and reduced mobility was reported for *G. pallida* by Storey (1984). He suggested measuring lipid reserves may enable population thresholds of *Globodera* to be altered for economic cropping of potatoes with rotational control. Our modeling projections suggest variation in lipid levels may influence the effective P_i of a population.

The population model suggests only cv. Gendarme and cv. Maria Huanca of 25 cultivars studied by Holz et al. (1999) would prevent buildup of damaging populations of *G. rostochiensis* with either a 3 or 4-year rotation at Toralapa (Table 2). Rotations of this duration are likely to result in damaging populations when the popular cultivar Waycha is planted. Even higher populations are likely if cv. Kosi is grown. Such predictions for the common cultivars like Waycha and Imilla Blanca are consistent with the high densities of potato cyst

nematodes that prevail in Bolivia (Franco et al., 1999). Extending rotations to limit damage is not always a feasible option. It is not possible if it requires a lower fraction of a land holding to be cropped each year than can provide the potatoes needed for subsistence living.

The cultivars of lupin and quinoa that enhance loss of eggs from cysts of *G. rostochiensis* (Franco et al., 2000) provide one basis of enhancing the overall population decline rate between potato crops, and transgenic resistance offers a second approach of high potential (Atkinson et al., 2001). The enhanced population model suggests that cultivar choice can also reduce current losses of potato due to *Globodera* spp. in Bolivia. Both these options are available to growers. The lack of usage of these options suggests farmers perceive such approaches have disadvantages. The population model also provides a basis for predicting the impact of cultivars offering partial resistance to the nematode.

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