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Gene flow as a factor in the evolution of insecticide resistance

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GENE FLOW AS A FACTOR IN THE EVOLUTION

OF INSECTICIDE RESISTANCE

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Abstract

The importance of gene flow in maintaining genetic variation among local populations in heterogeneous habitats has been debated for natural populations as well as populations subject to selection in manipulated ecosystems such as agricultural fields. The diamondback moth, Plutella xylostella (L.), is a pest of cruciferous crops and frequently treated with insecticides. Despite the high dispersal capabilities of this moth, considerable local variation exists in its response to insecticides, suggesting that gene flow among subpopulations may be restricted. The central question addressed by this study is whether this local variation is due to restricted gene flow, or if variation in selection due to different pesticide strategies is sufficient to maintain the variation.

Electrophoretic measurements of allozyme variation among 11 subpopulations on three islands in Hawaii indicate small amounts of genetic differentiation between subpopulations (overall F_{ST} estimates ranged from 0.038 to 0.028). This suggests that roughly 8 migrants are exchanged per subpopulation per generation. Smaller amounts of differentiation were observed between islands and between Hawaii and two continental populations, perhaps because insufficient time has passed for these larger population subunits to differentiate via drift.

Simulation of resistance evolution among treated and untreated fields in the diamondback moth suggests that migration rates greater than 10% are required before emigration from untreated into treated fields will delay resistance

evolution. Low rates of gene flow (<0.1%) delayed the overall rate of resistance evolution when resistance alleles were rare initially. The fastest rates of resistance evolution occurred at intermediate gene flow rates.

The effect of gene flow on the establishment of a pesticide resistant parasitoid, Trioxys pallidus, is examined in Chapter 3. Increased migration, either by dividing the number of resistant parasitoids released into smaller groups, or by increasing the migration rate, decreased the time required to implement the new strain. The simulations indicate that at least three years will be required for establishment of the resistant strain. The results demonstrate that implementation of genetically altered insects may require a significant proportion of the time allocated to a genetic manipulation project. Studies to identify potential problems in the implementation phase should be part of the initial consideration of any such project.

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Abstract

Gene flow and movement among Hawaiian populations of the diamondback moth, Plutella xylostella (L.), were examined using indirect and direct methods. Estimates of genetic differentiation of subpopulations (F_{ST}) based on electrophoretic variation at four polymorphic loci varied from 0.038-0.028, depending on the method used. Using Wright's method, estimates of the number of migrants exchanged per generation ($N_e m$) from F_{ST} estimates based on all loci ranged from 6.3 to 10.6. There was little variation between islands, and only slightly more variation between Hawaiian populations and two populations from the continental United States ($F_{region\text{-}total}$ = 0.011). The results are consistent with the recent arrival of the diamondback moth in Hawaii. There has probably been sufficient time since its introduction for local populations to have differentiated, but it is less likely that enough time has passed for larger population units to differentiate from each other. Results from field experiments in an area surrounding a cabbage plot suggest that diamondback moth is highly mobile. Average moth density in the 900m^2 surrounding the plot was 7.8% of the density of moths within the plot, indicating substantial emigration out of the plot. The

data indicate that local variation in insecticide resistance among Hawaiian diamondback moth populations is not an indication of restricted gene flow, and is most probably a result of local variation in selection.

Introduction

Local populations of the diamondback moth, Plutella xylostella (L.), in Hawaii show significant differences in insecticide resistance, even among populations separated by as little as 5km (Tabashnik et al. 1987). The wide variance in resistance among these populations suggests that there is little gene flow among populations. The diamondback moth, however, is known to migrate distances on the order of 3000km (Chu 1986, Lorimer 1981, Mackenzie 1958). P. xylostella would seem, therefore, to represent an insect with high potential for gene flow. An alternative hypothesis to explain the local variation in resistance is that gene flow occurs at a relatively high rate, but local variation in selection pressure, a result of different insecticide use by farmers, is high enough to maintain the variation between populations.

Estimates of gene flow from neutral or quasi-neutral allelic variation among populations can distinguish between these alternative hypotheses. High variation in allele frequencies among populations would indicate low gene flow between populations. This would support the hypothesis that local variation in insecticide resistance results from the low rates of movement of resistance alleles between populations. Low variation in allele frequencies would suggest that sufficient gene flow exists to move resistance alleles among populations.

Accurate estimates of gene flow depend on whether sufficient time has passed since the introduction of the diamondback moth into Hawaii to allow genetic drift within populations to reach a steady-state equilibrium with migration between populations. The diamondback moth was one of the first introduced species recorded in Hawaii and has been in the islands for over 100 years (Zimmerman 1978). In Taiwan, the diamondback moth breeds continuously, producing 15-20 generations per year (Chen and Su 1986). Climatic factors suggest that it also has 15-20 generations per year in Hawaii. Genetic similarity of local populations is unlikely to be the result of recent population fragmentation because more than 1500 generations have passed since the introduction of the moth. To the extent that differentiation between populations in Hawaii has not reached a steady-state, however, estimates of gene flow based on genetic differentiation will underestimate gene flow.

Computer simulation of resistance evolution in the diamondback moth among a matrix of treated and untreated fields suggested that resistance evolution in treated fields was not slowed by immigration from untreated fields unless the migration rate exceeded a critical value of about 10% (Chapter 2). Low migration rates ($\ll 0.1\%$) delayed resistance evolution when resistance alleles were initially rare because of the time required to move resistance alleles between populations.

Electrophoretic data estimate gene flow averaged over long periods of time (Slatkin 1987). Use of direct methods, such as mark-recapture, can measure individual movement during one time interval. Gene flow, however, may occur more commonly during catastrophic events, such as drought or destruction of host plant populations by alternative herbivores, which encourage migration. Mark-recapture studies which fail to include such events may underestimate dispersal. Measurements of individual movement, such as emigration from a field, may otherwise tend to overestimate gene flow because it is necessary for emigrants to locate new fields and reproduce there before they will actually contribute to gene flow (Ehrlich and Raven 1969, Endler 1979, Slatkin 1987). High rates of emigration, combined with high estimates of gene flow from electrophoresis, would suggest, however, that gene flow is occurring at the present time, and is not an artifact of past migratory episodes.

The objectives of this study were to obtain estimates of gene flow and movement of diamondback moths between populations in Hawaii using both an indirect method based on electrophoretic variation between populations, and a direct method based on the capture/recapture of diamondback moths emigrating from a cabbage field. The use of mark-recapture techniques also allowed estimates to be made of the adult population size in the cabbage fields examined.

Materials and Methods

Electrophoresis

Larvae of the diamondback moth were sampled from 13 locations, six on Oahu, four on Maui, one on Hawaii and one each from Wisconsin and Florida. Larvae were reared in the laboratory to the fourth instar on cabbage leaves, and frozen at -80°C for subsequent analysis. Average sample size for the Hawaiian populations was 48 individuals per locus, and ranged from 24 to 80. The populations OA1, OA2, OA3 and HW1 were collected from small, permanently established, untreated cabbage plots. OA4 and OA5 were collected from commercial watercress (Nasturtium officinale) farms. Population OA6 and all of the Maui populations were collected from commercial farms. The two populations from the continental United States were received as eggs from populations held in the laboratory for two generations. Two laboratory maintained populations were also examined. These populations had been established initially with populations of at least 50 individuals, and the average population size maintained in the laboratory exceeded 500 for most generations. These colonies had been maintained for approximately 75 generations (LAB1) and 35 generations (LAB2) when examined electrophoretically. LAB1 was established from a commercial farm on Maui near population MU4, while LAB2 was collected at an Oahu watercress farm approximately 2km from population OA4.

Individual larvae were prepared for electrophoresis by homogenization in 25μl of 0.2M Tris HCL with 0.5% mercaptoethanol and centrifuged for 1 min. Whatcom #3 paper wicks (2x4mm) were used to absorb the supernatant and placed into horizontal starch gels (10% Sigma and 2% Connaught starch). Gels ran 4-8 hours depending on the buffer system and were stained and scored immediately. A preliminary survey of 27 loci revealed eleven polymorphic loci, but of these only four were consistently resolvable and used in this study: mannose phosphate isomerase (MPI), malate dehydrogenase (MDH), phosphoglucose mutase (PGM), and phosphoglucose isomerase (PGI). The buffer systems used to resolve the loci were: LiOH for MPI (Selander et al. 1971), TC6.9 for PGM and MDH (Shaw and Prasad 1970), and TS6.0 for PGI (Shaw and Koen 1968). Alleles were labelled alphabetically with A as the allozyme which ran most anodally. Larvae from one of the laboratory maintained population were included as standards on each gel so that allozymes could be reliably identified across gels.

Mark-Recapture

Adults and larvae were collected at the Waimanalo Experiment Station, Waimanalo, Oahu. Larvae were reared to adulthood on cabbage and combined with field-collected adults. Adult F_1 progeny of the field collected moths were marked on the wings with Sharpie® permanent markers. Different colors were used on alternate releases. One hundred moths were placed into a 465ml container with a honey water source, and released into the center of a cabbage plot approximately 1hr before sunset. A total of 4125 moths were released in three different releases of 620, 1300 and 2205 moths.

The release site consisted of a central 140 plant cabbage plot surrounded by 30m of fallow ground to each side. Beyond this fallow ground were dirt roads and additional fields to the north and south, a hedge row to the east, and another fallow field to the west. The cabbage plants were arranged in seven rows of 20 plants, with 0.5m spacing between plants. Forty-nine pheromone traps (Trece pherocon 1C), placed 10m apart in a 7x7 square grid and on poles 0.5m above ground, were used to capture moths. Because the active space of these pheromones is less than one meter (Ishii et al. 1981), traps spaced 10m apart should not interfere with each other. The central trap of the grid was located in the cabbage patch; all others were in the fallow field. Diamondback moth pheromone lures (Raylo Chemicals, Edmonton, Canada) were replaced when moths were released. Pheromone traps were checked on alternate days for two weeks following a release and the number of marked and unmarked moths recorded. Weather parameters, including solar radiation, wind direction and wind speed, were recorded on a CR-10 data logger (Campbell Scientific, Logan, Utah).

Data Analysis

Genetic fragmentation between populations, a result of genetic drift of neutral alleles, will be limited by gene flow between the populations. Wright (1951) measured population differentiation as

$$F_{ST} = \frac{\sigma_p^2}{\bar{p}(1-\bar{p})}$$

where σ_p^2 is the variance in the frequency of an allele over all subpopulations and $\bar{p}$ is the average allele frequency over all subpopulations. Assuming an island model, in which a migrant from a population is equally likely to move to any other population, he noted that the average number of migrants exchanged by populations per generation could be estimated by:

$$N_e m \approx 1/4(1/F_{ST} - 1)$$

where N_e is the effective local population size and m is the average migration rate, or the proportion of a population which migrates per generation. The concept of F_{ST} has been extended to more than two alleles at a locus and multiple loci with the use of G_{ST} statistics (Nei 1973, 1977, Nei and Chakravarti 1977), and by further revision of F_{ST} by Wright (1978). Weir and Cockerham (1984) provided an estimate of differentiation ($\hat{\theta}_W$) that corrects for differences in sample sizes for different populations. Slatkin and Barton (1989) compared three estimates of $N_e m$; estimates of F_{ST} (G_{ST} and $\hat{\theta}_W$), the private allele method (Slatkin 1985), and maximum likelihood estimates (Barton et al. 1983). For island models of dispersal, G_{ST} and $\hat{\theta}_W$ performed the best. The F_{ST} estimators also proved to be good estimators of N_em under other dispersal models (stepping-stone, lattice and continuum models).

Allozyme frequency data were analyzed using the BIOSYS-1 package (Swofford and Selander 1981). Weir and Cockerham $\hat{\theta}_W$ statistics were calculated by a program written by the senior author. Deviations from Hardy-Weinberg expectations were calculated for each locus in each population using the chi-square goodness-of-fit analysis. This test will reject the null hypothesis too frequently when expected cell frequencies fall below five (Sokal and Rolf 1981). Therefore, if the chi-square goodness-of-fit test was significant and more than 20% of the cells had an expected frequency of less than five, we pooled genotype frequencies over the two allozymes with the lowest frequencies. This procedure was repeated until the above criterium was met, or until only two allozyme classes remained. In the latter case, a pooled comparison between the homozygotes of the most common allozyme, most common allozyme/all other allozyme heterozygotes, and all other genotypes was made (Swofford and Selander 1981).

G_{ST} and $\hat{\theta}_W$ were estimated for each locus, as well as over all loci. The populations from Wisconsin and Florida were excluded from F_{ST} estimates used in calculating N_em. Variance in the overall $\hat{\theta}_W$ value was estimated with a jackknife procedure (Weir 1990). F_{IS} values, which measure deviations from random mating within subpopulations, were computed for each allele at all loci and compared to 0 using $X^2_{(1)} = N*F^2_{IS}$ (Workman and Niswander 1970), where N is the number of individuals sampled at the locus. Genetic distances between populations were calculated using both Nei's original (Nei 1972) and unbiased method (Nei 1978). Genetic distance data were reduced using multidimensional scaling (Wilkinson 1988), which placed populations along two axes so that the metric distance between any two populations was roughly proportional to the genetic distance between those populations. This method is preferable to dendrogram construction in this case because it does not presume any historical relationships among the populations.

In the mark/recapture experiments, the relative adult density per unit area was estimated directly from the number of captures per trap. The dropoff of density of captured moths from the release point with distance (assuming that all moths, both marked and unmarked, originate from the cabbage plot), can then be calculated using the general equation given by Taylor (1978):

$$N = e^{\eta + bX^c}$$

where N is the number of recaptures for a trap, X is the distance of the trap from the center, and η, b and c are constants estimated by nonlinear regression (Wilkinson 1988). Taylor (1978) suggests that if dispersal is random, then the estimate for the value of c should approximate 2. Lower values indicate a tendency to aggregate about the release point.

Moth recapture rates can be used to estimate adult population size. Because there was an established population in the cabbage plot, the recapture rate over time would tend to overestimate population size. The number of unmarked moths could be assumed to be constant, while the number of marked moths declined due to mortality and emigration. To estimate population size, we therefore used only the recapture data from the first collection period, on the second day following the release.

Results

Electrophoresis

Allele frequencies for the 15 populations are shown in Table 1.1. There were significant deviations from Hardy-Weinberg expectations at loci within three different populations, out of a total of 60 comparisons (OA4/PGI, $p=0.002$; HW1/PGI, $p=0.035$; MU1/MDH, $p=0.032$). While this number of significant comparisons does not exceed the number expected by chance alone, two of these comparisons were at the PGI locus, and in both cases there was a deficiency of heterozygotes. The chi-square results suggest there were no significant deviations from random mating within populations with respect to the other three loci. F-statistics assume that loci are neutral. The PGI locus deviated from

Table 1.1. Allozyme frequencies from 15 populations of P. xylostella at four polymorphic loci. Collection regions: LA# - Laboratory reared colonies, OA# - Oahu, HW# - Hawaii (Island), MU# - Maui, FL1 - Florida, WI1 - Wisconsin.

	Population														
Locus/ Allele	LA1	LA2	OA1	OA2	OA3	OA4	OA5	OA6	HW1	MU1	MU2	MU3	MU4	FL1	WI1
MPI(N)	38	49	49	29	48	58	69	63	46	30	43	38	44	29	32
A	.000	.000	.000	.017	.000	.000	.007	.000	.000	.000	.000	.000	.000	.000	.000
B	.158	.184	.133	.069	.021	.078	.094	.048	.054	.050	.047	.053	.068	.034	.031
C	.197	.235	.469	.362	.448	.397	.312	.381	.326	.383	.372	.421	.398	.155	.281
D	.289	.531	.214	.362	.500	.371	.319	.460	.457	.433	.442	.382	.398	.448	.453
E	.342	.031	.163	.138	.031	.155	.225	.087	.130	.133	.116	.132	.091	.362	.234
F	.013	.020	.029	.052	.000	.000	.044	.000	.033	.000	.023	.013	.045	.000	.000
PGM(N)	79	59	59	23	78	32	39	64	50	30	46	40	58	31	32
A	.000	.017	.000	.000	.006	.000	.064	.000	.000	.000	.000	.000	.009	.000	.000
B	.108	.390	.085	.000	.051	.063	.077	.102	.070	.067	.065	.075	.086	.000	.000
C	.361	.254	.534	.761	.692	.656	.474	.766	.600	.667	.641	.712	.724	.661	.922
D	.158	.144	.314	.239	.128	.234	.269	.117	.310	.233	.293	.188	.155	.306	.078
E	.373	.195	.068	.000	.122	.047	.115	.016	.020	.033	.000	.025	.026	.032	.000
MDH(N)	59	50	67	24	80	43	42	47	49	31	43	34	54	24	22
A	.000	.000	.000	.000	.006	.000	.083	.000	.020	.000	.023	.044	.000	.000	.000
B	.432	.490	.903	.917	.906	.523	.798	.777	.888	.758	.895	.868	.880	.979	1.00
C	.568	.510	.097	.083	.087	.477	.119	.223	.092	.242	.081	.088	.120	.021	.000
PGI(N)	100	83	61	36	75	54	59	46	51	34	46	50	57	31	32
A	.020	.048	.033	.069	.020	.028	.008	.011	.196	.103	.054	.040	.009	.000	.000
B	.250	.223	.180	.069	.187	.120	.271	.185	.353	.132	.152	.120	.193	.081	.031
C	.550	.554	.730	.833	.580	.574	.568	.620	.324	.632	.652	.700	.632	.919	.969
D	.165	.133	.049	.028	.187	.176	.076	.087	.108	.132	.141	.140	.149	.000	.000
E	.010	.042	.008	.000	.027	.083	.051	.098	.020	.000	.000	.000	.018	.000	.000
F	.005	.000	.000	.000	.000	.019	.025	.000	.000	.000	.000	.000	.000	.000	.000

Hardy-Weinberg expectations more often than would be expected by chance alone. F_{IS} (Table 1.2) is a measure of the degree of random mating within populations, and it should approach zero when mating is random within populations. The F_{IS} value for PGI was significantly different from 0 at five of six alleles for the PGI locus, and the overall estimate also exceeded 0.1. This suggests the possibility of the presence of a disturbing force such as selection or assortative mating at this locus. Because the F_{IS} values are not uniformly high across all loci, the deviations are unlikely to be the result of the Wahlund effect (lumping disparate population subunits into single sampling units), which would tend to affect all loci alike. The Wahlund effect would cause a heterozygote deficiency and raise the F_{IS} estimates. Selection and assortative mating could also be responsible for deviations from Hardy-Weinberg expectations, and would affect single loci only. Our data, therefore, are consistent with the possibility that selection has influenced the allele frequencies at the PGI locus. F_{ST} measures, however, are robust against certain types of selection as long as the selection coefficient is not much larger than the migration rate (Slatkin and Barton 1989). Because the F_{ST} estimates for the PGI locus were consistent with estimates from the other loci, we included data from this locus in the remainder of the analysis.

Estimates for the F-statistics (Table 1.2) indicate little genetic differentiation between islands or between populations ($F_{ST} < 0.05$). Overall estimates for N_em range from 6.3 - 10.6 (Table 1.2), depending upon the method used to estimate F_{ST}. Hierarchical F-statistics comparing within-island

Table 1.2. F-statistics summary from 11 Hawaiian populations of P. *xylostella*.

Locus	F_{IS}	G_{ST}[A]	$\hat{\theta}_W$[B]	W_{78}[C]
MPI	0.033	0.016	0.008	0.006
PGM	-0.016	0.029	0.022	0.018
MDH	0.032	0.090	0.077	0.082
PGI	0.132	0.047	0.035	0.016
MEAN	0.048	0.038	0.029	0.028
σ^2	--	--	0.0015[D]	0.057
Overall N_em estimate		6.32	8.37	10.6

[A]Nei (1977)

[B]Weir and Cockerham (1984)

[C]Wright (1978), Swofford and Selander (1981)

[D]Estimated by the jackknife procedure over loci variation to between-island variation (BIOSYS-1, STEP WRIGHT78), indicated little differentiation between islands ($F_{population\text{-}island}$ = 0.028, $F_{island\text{-}region}$ = -.001).

F-statistics comparing the Hawaiian populations to the two mainland populations examined also suggested that there was little differentiation between mainland and Hawaii populations ($F_{population\text{-}region}$ = 0.034, $F_{region\text{-}total}$ = 0.011).

Genetic distances between field populations in Hawaii (Table 1.3) averaged 0.04 ± 0.004. The average genetic distance between the two laboratory populations and Hawaiian populations was 0.21 ± 0.01, and the average distance between the two mainland populations and Hawaiian populations was 0.066 ± 0.008 (Fig. 1.1). One Oahu population collected from watercress (OA4) differed substantially from all other Oahu populations as well as populations from other islands and the mainland.

Mark-Recapture

A total of 597 moths were captured in the pheromone traps, of which 14 were marked. Assuming that half of the released moths were males, the total recapture rate was 0.67%. Averaged over the three releases, the estimated adult population size was 2711 ± 791, which agrees well with a previous estimate of 2489 ± 386 (Caprio, unpublished data). The distribution of moth captures (Fig. 1.2) was skewed to the south. The predominant wind direction at

Table 1.3. Nei's genetic distance between 15 diamondback moth populations. Above the diagonal are unbiased genetic distances (Nei 1978), below are original genetic distances (Nei 1972). Populations were collected from: LB# - laboratory colonies, OA# - Oahu, HW# - Hawaii (Island), MU# - Maui, WI - Wisconsin, FL-Florida.

	LB1	LB2	OA1	OA2	OA3	OA4	OA5	OA6	HW1	MU1	MU2	MU3	MU4	WI	FL
LB1	--	0.09	0.21	0.25	0.24	0.09	0.15	0.19	0.27	0.16	0.23	0.22	0.22	0.24	0.29
LB2	0.10	--	0.22	0.25	0.20	0.12	0.17	0.16	0.23	0.14	0.20	0.21	0.19	0.27	0.30
OA1	0.22	0.23	--	0.02	0.04	0.09	0.01	0.05	0.07	0.03	0.02	0.02	0.02	0.05	0.06
OA2	0.26	0.26	0.03	--	0.03	0.08	0.03	0.02	0.08	0.01	0.01	0.00	0.01	0.02	0.01
OA3	0.25	0.21	0.05	0.04	--	0.08	0.04	0.01	0.04	0.01	0.01	0.01	0.00	0.08	0.05
OA4	0.11	0.13	0.11	0.09	0.09	--	0.08	0.03	0.12	0.02	0.07	0.06	0.06	0.13	0.12
OA5	0.17	0.18	0.02	0.05	0.05	0.10	--	0.04	0.04	0.02	0.01	0.02	0.02	0.04	0.07
OA6	0.20	0.17	0.06	0.03	0.02	0.05	0.05	--	0.06	0.00	0.02	0.01	0.00	0.07	0.04
HW1	0.28	0.24	0.08	0.09	0.05	0.14	0.05	0.07	--	0.06	0.03	0.05	0.04	0.11	0.13
MU1	0.17	0.16	0.04	0.02	0.02	0.04	0.04	0.01	0.04	--	0.00	0.00	0.00	0.05	0.04
MU2	0.24	0.21	0.03	0.02	0.02	0.08	0.03	0.02	0.04	0.01	--	0.00	0.00	0.03	0.04
MU3	0.24	0.22	0.02	0.01	0.02	0.08	0.03	0.02	0.07	0.01	0.01	--	0.00	0.04	0.02
MU4	0.23	0.20	0.03	0.02	0.01	0.07	0.03	0.01	0.05	0.01	0.01	0.01	--	0.05	0.03
WI	0.25	0.28	0.05	0.03	0.08	0.15	0.05	0.08	0.12	0.06	0.04	0.05	0.06	--	0.02
FL	0.30	0.31	0.07	0.02	0.06	0.13	0.08	0.04	0.13	0.05	0.04	0.03	0.04	0.03	--

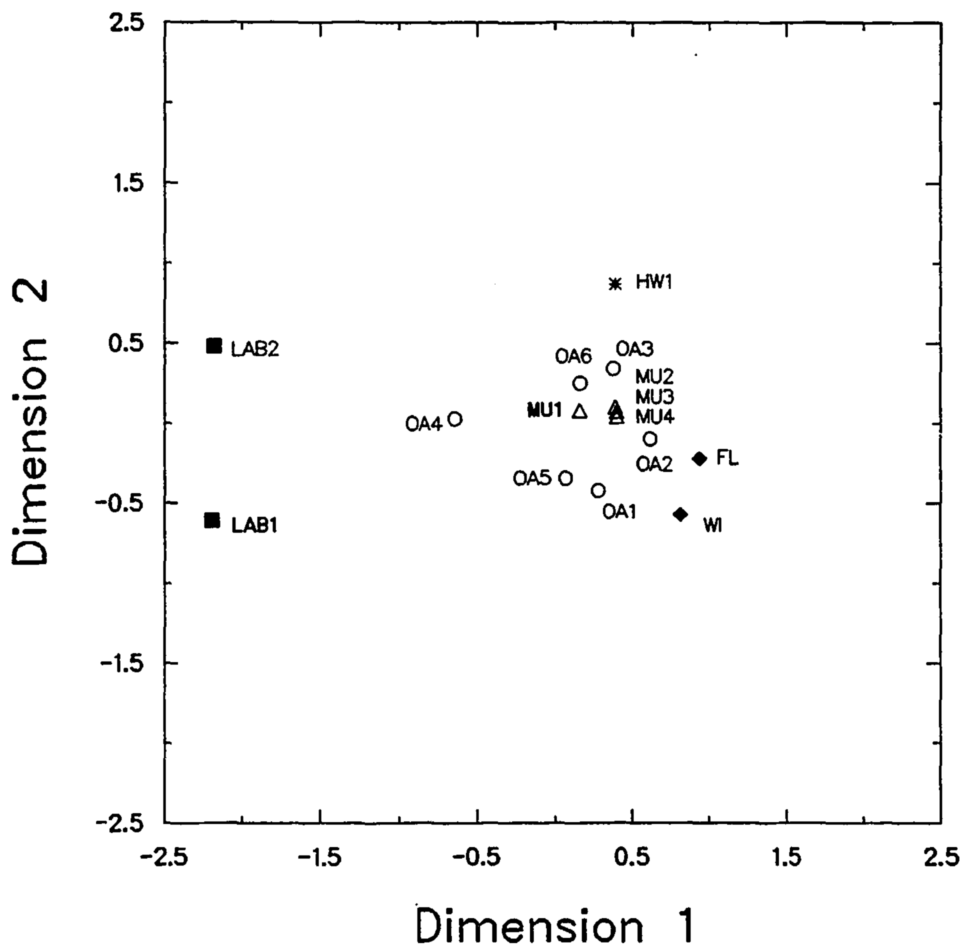

Figure 1.1. Genetic distance between 15 diamondback moth populations. The metric distance between any two populations is proportional to the genetic distance between the populations.

the field site was from the north. Thus the moths disperse primarily downwind. 125 moths were captured in the single trap in the center of the cabbage plot, while an average of 9.8 moths were captured in traps outside of the plot. Nonlinear regression of the number of captures vs. distance using Taylor's equation (Fig. 1.3) estimated the parameter c to be -0.63, indicating that the moths did not disperse randomly from the release point but tended to aggregate in the cabbage plot.

Discussion

Results from electrophoresis and mark/recapture experiments suggest that substantial movement occurs between diamondback moth populations in Hawaii. Estimates of $N_e m$ from the electrophoretic data range from 6.3-10.6 individuals exchanged per generation per population depending on the method used to estimate F_{ST}. While this rate of gene flow will not prevent significant allelic variation between populations (Allendorf and Phelps 1981), it is sufficient gene flow to move adaptively significant alleles relatively rapidly throughout populations. Local differentiation in resistance in the diamondback moth is therefore unlikely to be the result of restricted gene flow. Our results suggest that variation in resistance is the result of local differences in pesticide use and perhaps stochastic events occurring when resistance alleles are rare (Chapter 2).

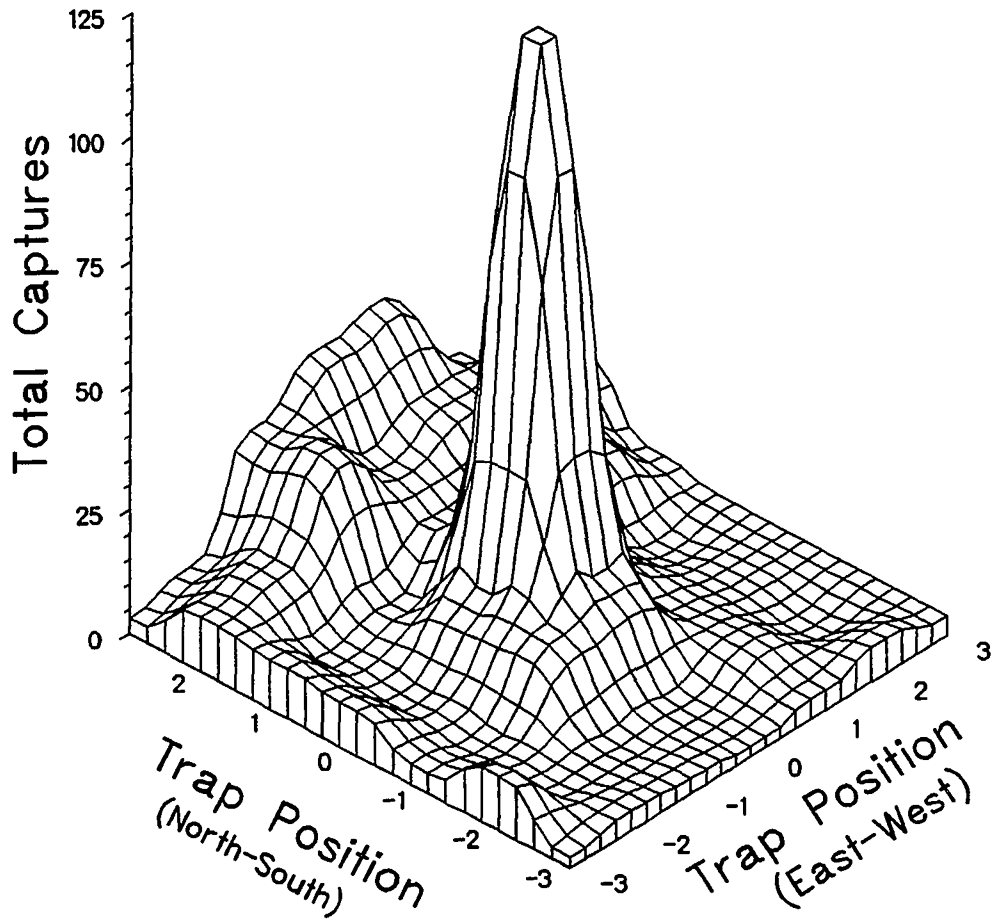

Figure 1.2. Total number of captures per trap over all three releases. The center peak covers the location of the cabbage plot, all other traps were located in a fallow field.

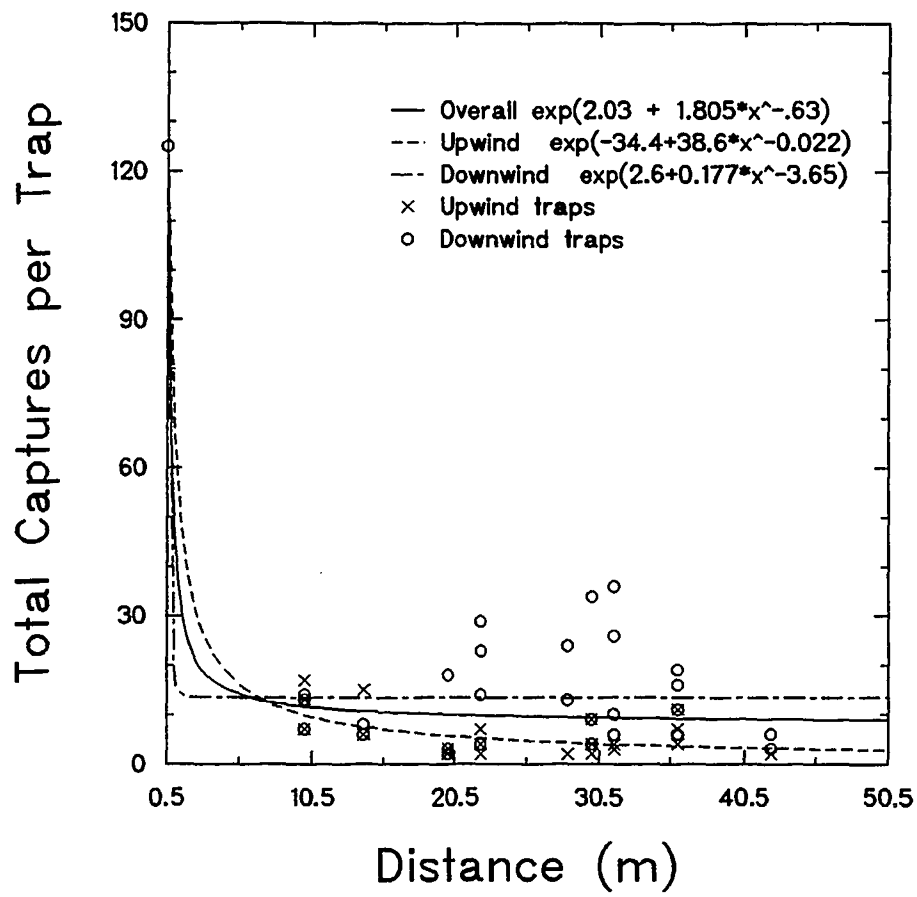

Figure 1.3. The dropoff in the number of captures with distance from the release site. The data is fit to Taylor's (1978) general equation for the overall captures and for recaptures divided into upwind and downwind traps.

Accurate gene flow estimates depend on whether sufficient time has passed since fragmentation to allow local differentiation to occur. The amount of time required is a function of both the local population size, N_e, and the migration rate. Crow and Aoki (1984) found the time required to go halfway to equilibrium in a stepping stone model was approximately $\ln(2)/[2m + 1/(2N)]$. Larger subpopulation units take longer to approach equilibrium because drift changes allele frequencies more slowly among these subpopulations, while lower migration rates result in higher equilibrium F_{ST} values, which take longer to approach. Assuming that the ratio of the population size to the number of plants remains constant across fields of different size, our estimates of diamondback moth population size from the small mark/release plots suggest that the population sizes in the fields sampled for electrophoresis varied from roughly 5000 to 20,000 adults. Differences in reproductive success may lower the effective population size by 90% (Wright 1978). Assuming our measures of population size overestimate effective size by only a factor of two, and estimate gene flow conservatively at $N_em=5$ and $N_e=10{,}000$, it would take approximately 660 generations for F_{ST} to approach half its equilibrium value. The diamondback moth has presumably gone through at least three times as many generations in Hawaii since its initial introduction. On the other hand, if gene flow between the islands of Hawaii, or between the mainland and Hawaii, is restricted, and the population units examined are larger, then much more time will be required before F-statistics at those levels will reach equilibrium values. If the migration rate between islands is just one-tenth the rate between populations within an island, it would take at least 4620 generations for F_{ST} to approach half its equilibrium value. We suggest that an inadequate amount of time has occurred for significant differentiation to have developed between islands and between Hawaii and the mainland, and that this responsible for the low values of the hierarchical F-statistics at these levels, rather than high rates of gene flow between islands. The differentiation observed between Hawaii and the mainland may be a product of founding events, or it may be that North America was not the source for introductions of the moth into Hawaii. These data illustrate a potential misunderstanding in the use of F-statistics. Their use in the study of recently established populations must be carefully qualified with the observation that different levels of population substructures may approach equilibrium state at different rates depending on the population size and migration rate. Thus, higher levels in population hierarchies (e.g., islands vs. populations within islands), may often show lower levels of differentiation for longer periods of time following fragmentation because of their larger population size, and this should not be construed as being the result of higher rates of gene flow between these larger subunits of the total population.

The low rates of differentiation between populations are not likely to be the result of genetic constraints related to selection (negative genetic correlations or epistatic interactions, for example), because the two populations which were brought into the laboratory and held isolated for at least 40 generations were substantially differentiated from the field populations and, to a lesser extent, from each other.

The mark/recapture field experiments also indicated substantial movement of the diamondback moth out of cabbage plots. The estimate of c from Taylor's equation indicates that the moths tended to aggregate around the release point, but the average density of moths in the fallow field surrounding the cabbage plot was still 7.8% of the density of moths in the plot. The large percentage of moths captured outside of the field suggest that a minimum of 7.8% of the population emigrated per day. This emigration rate cannot be used to estimate gene flow among populations because the experiment addressed neither the success of these emigrants in locating new fields, nor their reproductive success once a new population was found, both additional requirements for gene flow to occur. Thus, though these experiments cannot be used to provide a precise estimate of gene flow, the results suggest the potential for substantial gene flow between populations and agree qualitatively with the electrophoretic data.

These results demonstrate that substantial gene flow and movement occurs among diamondback moth populations in Hawaii. Our estimates of gene flow in this species indicate that it is neither high enough to delay resistance evolution due to the immigration of susceptible moths from untreated populations, nor is gene flow so low that substantial delays in resistance evolution would occur because of the slow spread of initially rare alleles. We suggest, therefore, that the local variation observed in resistance levels does not reflect restricted rates of gene flow. The results suggest that gene flow has been substantial, yet it has not been high enough to overcome local variation in selection resulting from variation in use of pesticides.

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Chapter 2. Local Adaptation and Gene Flow Among Finite Populations: Simulation of Evolution of Insecticide Resistance

Abstract

Stochastic computer simulation of the evolution of insecticide resistance among finite, subdivided populations showed that interactions among gene flow, population size, initial genetic variation, and environmental heterogeneity influence adaptation to local selection. When all subpopulations were exposed to pesticide selection, gene flow had little effect unless the allele providing resistance was rare (the product of the initial resistance allele frequency and population size was less than 20). When rare, the resistance allele was lost in many of the finite subpopulations. Thus, higher rates of gene flow increased the overall rate at which resistance evolved by spreading the resistance allele more rapidly from subpopulations where it had persisted. In heterogeneous environments where the resistance allele was at a selective advantage in treated subpopulations, but at a slight disadvantage in untreated subpopulations, intermediate levels of gene flow produced the most rapid rates of local adaptation. At higher rates of gene flow, migration of susceptible alleles into treated subpopulations delayed the evolution of resistance. At low rates of gene flow, resistance evolution was delayed by the low rate of movement of resistance alleles between subpopulations. Although high gene flow ($>10\%$ per generation)

retarded resistance evolution in treated subpopulations, it greatly increased the resistance allele frequency in the untreated subpopulations. Results from finite population models suggest that in most field situations, gene flow promotes evolution of insecticide resistance. Previous results from infinite, continent-island population models had supported the opposite conclusion. These qualitatively different conclusions illustrate the importance of finite population size, particularly when lack of genetic variation constrains evolutionary adaptation.

Introduction

Gene flow among populations in different habitats affects the maintenance of polymorphisms that reflect local adaptation to different selection regimes. Gene flow is not a force per se in this regard, but rather arbitrates between local and global selection (Spieth 1979). Haldane (1930) demonstrated a selection-migration balance in a continent-island model with migration only from the continent to the island. The locally adaptive allele on the island persisted only when migration fell below a rate determined by the selective advantage of the adaptive allele on the island. Other models of environmental heterogeneity also suggest that local adaptation will occur if migration falls below some critical value (Gillespie 1975, Spieth 1974, 1979, Pollak 1974).

In contrast, Takahata (1983) found that intermediate gene flow levels resulted in the highest level of genetic variance among populations when there was random variation in selection in an island model. If gene flow was too high, the population behaved as if it were panmictic, and no local adaptation occurred. If gene flow was too low, mutant alleles were not transported between populations and genetic variance decreased.

The evolution of resistance to insecticides among mosaics of treated and untreated fields is a special case of local adaptation. Because selection is exceptionally strong in this case, models of resistance are generally concerned with the rate at which resistance evolves rather than the maintenance of polymorphism as in most population genetic models (eventual fixation of the resistance allele is generally conceded at the onset). Nevertheless, parallels exist to other population genetic models. Models have shown that evolution of insecticide resistance depends primarily on selection intensity, degree of dominance, size of refugia (escape from insecticide selection by some individuals) and gene flow (Comins 1977a, 1977b, 1979, Georghiou and Taylor 1977a, 1977b, Plapp et al. 1979, Taylor and Georghiou 1979, Tabashnik and Croft 1982, Tabashnik 1986). Selection experiments with laboratory populations have confirmed the importance of the degree of dominance of the resistance allele and migration rate (Prasittisuk and Curtis 1982, Taylor et al. 1983, Flexner et al. 1989).

The ability of gene flow to retard local adaptation to insecticide treatments has been demonstrated in several simulations (Georghiou and Taylor, 1977a; Taylor and Georghiou 1979, Tabashnik and Croft 1982), all of which used a continent-island model. In contrast, Comins (1977a, 1977b) examined a two-field system (one field treated with insecticide and one field untreated). Although migration in this system retarded resistance evolution in the treated field, it increased the rate of resistance evolution in the untreated field. Results from a multilocus model suggested that migration might reduce pleiotropic costs associated with resistance (Uyenoyama 1986). In this model, migration increased the effective population size and genetic variability of treated populations. At the same time, migration preadapted untreated populations by spreading alleles that promoted the separation of new pesticide detoxification pathways from their original metabolic functions.

Many of the models discussed above ignore particulate inheritance and the stochastic nature of evolution in finite populations. Particulate inheritance implies that alleles cannot exist as fractional units. As a result, alleles in substructured populations, especially rare ones, may be lost by chance in one or more subpopulations. The objective of our research was to determine how stochastic factors influence the effects of migration on the evolution of resistance among multiple finite populations. Stochastic factors are likely to be important because resistance alleles are thought to exist at low frequencies prior to selection with insecticides (10^{-3}-10^{-13}, Roush and McKenzie 1987). At such low frequencies, the fate of rare resistance alleles may be affected by random events even when the effective population size is large.

We used stochastic simulation to address three specific objectives: 1) to determine how gene flow affects local adaptation (resistance evolution) when all fields are treated and all populations initially have a relatively low frequency of a selectively advantageous allele; 2) to examine how environmental heterogeneity (a matrix of treated and untreated fields) interacts with gene flow and dominance to affect local adaptation; and 3) to measure the interaction between the initial resistance allele frequency and population size.

Materials and Methods

Simulation Framework

Independent population growth was simulated in each of either 25 or 49 fields arranged in a square matrix. Each field was equivalent to an ecological patch of homogeneous habitat. Habitat types were defined by different demographic parameters for the resident population, including mortality, reproductive rate, developmental rate, and dispersal rate for each life stage (egg, larva, pupa and adult) of the insect as well as the rate of pesticide application. The model was similar to a nonlinear, multiregional, fixed stage duration Leslie matrix (Caswell 1989) with insects classified by field, age (days within stage), sex, and genotype. In addition, females were classified by the genotype of their last mate. Unlike a standard Leslie matrix model, the coefficients of the transition (A) matrix were parameters of stochastic probability functions rather than constants. The state matrix at time t+1 was a function of a random draw from the probability distribution and the state matrix at time t (Pollard (1966, 1973) discusses a similar linear model with unvarying environment). Mortality, reproduction, mate genotype, and dispersal were determined stochastically, allowing for random events and genetic drift (see Appendix A for a detailed description of the specific algorithms used). Populations within fields were finite and inheritance was particulate, so that both the state matrix and number of alleles in a field consisted only of integers. Each simulation was replicated three times.

Density-dependent population regulation was achieved with the Verhulst-Perl (logistic) equation (Spain 1982). A daily multiplicative reduction in reproductive output was calculated from the population density, which included all of the defined life stages (egg, larval, pupal and adult). The carrying-capacity (k-value) was 100,000 individuals per field. All populations started with 80,000 individuals in a stable age distribution unless otherwise noted.

Dispersal

Dispersal was modeled as an open-ended random walk (stepping-stone with multiple steps possible) defined by two parameters. The number of insects from each field that dispersed at time t was determined by a random draw from $b(N_t, p_1)$, a binomial distribution given by N_t (the number of adults in the field at time t) and p_1, the chance that an individual will disperse per day. Each insect that dispersed then started out on a random walk, the length of which depended on the second dispersal parameter, p_2. On each step of the random walk, the insect randomly moved into one of the eight adjacent fields, and a second uniform random number was compared to p_2. If greater, the insect stopped, otherwise the process was repeated from the new field. P_2 was set at 0.1, so that 90% of dispersing insects stopped after moving one field (as would all individuals under a strict stepping-stone model). This model differs from a strict stepping-stone model because long-distance dispersal, while rare, is permitted. Movement was allowed to all eight adjacent fields, including those in the corner positions, rather than restricting movement to the four neighboring fields that share a full border. Individuals that moved out of the field system on a random walk were returned to their original field. This lowered the effective migration rate but maintained a constant mortality rate compared to an alternative model where insects moving out of the system died.

The total percentage of a population that disperses can be approximated by multiplying p_1 by the average life-span of the adult (ca. 6 days). This overestimates the reproductive potential of the dispersants, as young dispersants can expect to contribute more offspring in the new field than can adults which disperse later in life. Disregarding the low adult mortality rates during the reproductive period, the reproductive contribution of these older dispersants to the new field will be approximately half of the total reproductive expectation of those adults. Table 2.1 gives approximate equivalents to p_1 in both the percentage of population which disperses and the number of migrants exchanged per generation.

Table 2.1. Equivalent migration rates in both migrants per generation (Nm) and m (proportion of population exchanged per generation). The time adjustment adjusts raw dispersal rates for reproductive success in the new field (later dispersers reproduce less in the new field than early migrants). The field adjustment adjusts for migration between field types.

	Dispersal rate (daily migration rate)				
Migrants/generation	0.072	0.0143	0.00143	0.000143	0.0000143
Time adjusted	2520	500	50	5	0.5
Time and field adjusted	1260	250	25	2.5	0.25
Migration rate (per generation)					
Unadjusted	0.5	0.1	0.01	0.001	0.0001
Time adjusted	0.25	0.05	0.005	0.0005	0.00005
Time and field adjusted	0.125	0.025	0.0025	0.00025	0.000025

Life History

Life history parameters were based on Plutella xylostella (L.), the diamondback moth, a global lepidopterous pest of cruciferous crops (Table 2.2). The parameters used here are modifications of those used in the single field, deterministic, infinite population model of Tabashnik (1986). The present simulation was based on a one locus, two-allele model, where one allele conferred susceptibility and the second conferred resistance to pesticide. Resistance was showed partial dominance (i.e., the fitness of the heterozygotes was closer to the wild type homozygotes than the resistant homozygotes when treated with pesticide), except in those simulations where the dominance of the resistance allele was varied. Only immature stages were affected by insecticide treatments. Pleiotropic costs associated with resistance were reflected by a decrease in reproductive output of resistant moths (18.75 offspring/day vs. 23.75 for heterozygotes and 25 for wild-type moths). A background mutation rate of 1×10^{-6} per birth was included in the simulation.

Insecticide Treatments

Depending upon the experiment, all of the fields were periodically treated with pesticide, or a mixture of treated and untreated fields were maintained (but

Table 2.2. Demographic parameters used to simulate the diamondback moth (P. xylostella) life-cycle.

Stage	Egg	Larval	Pupal	Adult
1. Days in stage	3	6	4	7
2. Mortality	0.0054	0.31	0.155	0.05
3. Reproduction/female/day				
Resistant (RR)	--	--	--	18.75
Heterozygote (RS)	--	--	--	23.75
Susceptible (SS)	--	--	--	25.0
4. Dispersal (p_1)	0	0	0	0.071
5. Affected by pesticide	Yes	Yes	Yes	No

Response to Pesticide

	RR homozygotes	RS heterozygotes	SS homozygotes
LC_{50}	9.12	0.096	0.013
Slope	2.23	1.11	1.41

both used the same demographic parameters). Environmental heterogeneity caused by insecticide applications favored different alleles in the treated and untreated fields (fecundity selection favored the susceptible allele in untreated fields and mortality selection favored the resistant allele in the treated fields).

Treated fields were sprayed every 10 days with a dose of pesticide (0.191 ppm, units are relative to the lc_{50} and slope parameters) that killed 95% of the susceptible homozygotes (Table 2.2). The response to pesticide is based on the response by a Taiwanese population to a pyrethroid pesticide, fenvalerate (Liu et al. 1981). The pesticide decayed with a half-life of 3 days and was ignored after 7 days. Mortality of heterozygotes at the dose applied was 44%, except in those cases where the resistant allele was considered to be dominant (mortality due to insecticide was $< 1\%$).

Statistical Analysis

We measured the rate of resistance evolution among multiple fields in two ways. Time series were compared by measuring the average resistance allele frequency over all fields of one type (treated/untreated). If the population size in a field was less than 1000, we considered the field to be extinct, and assumed that the resistant allele frequency for that field was 0 (since no resistant alleles had yet established in the field). This eliminated transient increases in resistance levels caused by events such as resistant males migrating into an unpopulated field (where treatments had eliminated a susceptible population). While chance events such as these can be important, the population size limitation filters out those random events which do not result in permanent allele frequency deviations.

We also measured the rate of resistance evolution by recording the time it took for 50% of the fields to have a resistant allele frequency greater than 0.5. This approach resulted in a single value dependent on the rate of resistance evolution that was amenable to further testing by analysis of variance (ANOVA, Systat MGLH module, Wilkinson 1988). A natural log transformation was performed on this data prior to ANOVA to stabilize variances.

Experimental Design

Objective 1 - Gene Flow Among Homogenous Finite Populations

Each replicate of the experiment consisted of a 25-field system in which all fields were exposed to 60 insecticide treatments over 600 days (ca. 35 generations). A completely randomized 3x4 factorial design was used, with the initial resistance allele frequency varied over three levels (0.002, 0.0002 and 0.00002) and the gene flow rate (measured as the proportion of adults dispersing per day) varied over four levels (0.07, 0.0143, 0.00143, and 0.000143).

Objective 2 - Gene Flow Among Heterogeneous Finite Populations

Each replicate of this experiment consisted of a 49-field system, with 25 randomly selected treated fields and 24 untreated fields. A completely randomized 3x4 factorial design was used with three levels of initial resistance allele frequency (0.02, 0.002 and 0.0002) and four levels of gene flow (0.0143, 0.00143, 0.000143 and 0.0000143). In addition, the dominance of the resistance allele was varied from the standard incompletely recessive (see Table 2.2) to complete dominance. For the dominance model, the initial resistance allele frequency levels were $5x10^{-6}$, $2x10^{-4}$ and $2x10^{-2}$, and the gene flow rates were 0.071, 0.00143, 0.000143 and 0.0000143.

Objective 3 - Initial Resistance Allele Frequency and Population Size

To determine how the initial resistance allele frequency (IRF) and population size interact to affect resistance evolution, 25-field systems were established with no untreated fields and no gene flow between fields. The carrying capacity (k) was varied from 20,000 to 160,000, while the initial resistance allele frequency was varied so that the initial resistance allele frequency x population size product varied from 4 to 128 (this product is the average number of resistant alleles initially present in each field). Populations were initiated with population sizes equal to the carrying capacity at a stable age distribution. Because there was no gene flow, resistance could only develop in fields where there was sufficient local genetic variation. The number of fields that developed resistance was therefore a measure of the ability of fields to develop resistance without the influx of additional genetic material via gene flow.

Results

Gene Flow Among Homogeneous Finite Populations

In a system with 25 treated fields, when the initial resistance allele frequency was relatively high (0.002), the rate of resistance evolution was not significantly affected by gene flow (Fig. 2.1a, ANOVA, $F = 1.24$; $df = 3,8$; $P = 0.356$). Under these conditions, resistance alleles were initially common, and almost all fields developed resistance locally. When the initial resistance allele frequency was low (0.00002, Fig. 2.1b), higher levels of gene flow significantly increased the rate of resistance evolution (ANOVA, $F = 17.75$; $df = 3,8$; $P = 0.001$). When resistance alleles were rare, they were frequently lost and relatively few populations developed resistance locally. Other populations relied primarily on gene flow (mutation also generated variation but at a much lower rate) to provide the genetic variability to respond to the pesticide.

Gene Flow Among Heterogeneous Finite Populations

The addition of 24 untreated fields to the system did little to change the overall pattern of resistance evolution in the treated fields (Figs. 2.1b, 2.2b), though high rates of gene flow tended to retard resistance evolution. Under the incomplete dominance model with a high initial resistance allele frequency (0.02),

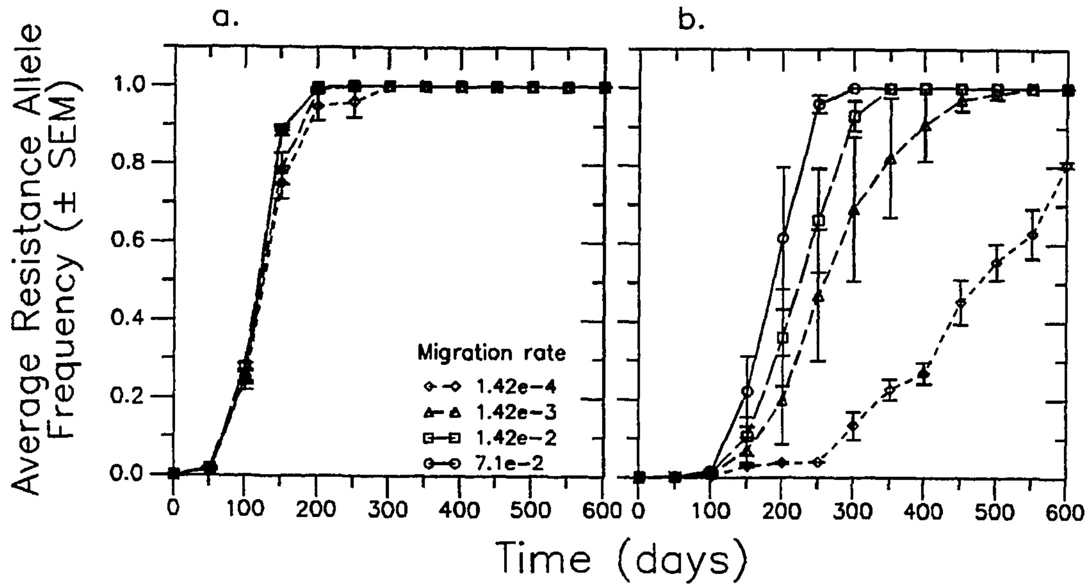

Figure 2.1. Effects of gene flow and initial resistance allele frequency on evolution of resistance in a homogeneous environment (all 25 fields treated with insecticide). Average resistance allele frequency among all fields is shown when the initial resistance allele frequency is: a. - High (0.002, average of 200 alleles per field); b. - Low (0.00002, average of 2 alleles per field). See Table 2.1 for details on migration rate.

the rates of resistance evolution were not significantly influenced by gene flow except at the high rates, where resistance was retarded (Fig. 2.2a). Because the model became increasingly deterministic as the initial resistance allele frequency increased, several of the gene flow treatment levels had no variance at the highest initial resistance allele frequency level, violating assumptions of both parametric (homogeneity of variance) and nonparametric (identical shape) analyses. It is possible to compare the three replicates of the high gene flow level (141, 141 and 161 days) to see if the mean value is significantly different from 91 days, the value of all six replicates at the two low gene flow rates (but for which we have no variance estimate). To keep the experimentwise error rate at 0.05 when choosing one out of six possible pairwise comparisons, we set the significance level at 0.0083. The rate of resistance evolution at the high gene flow level differed significantly from 91 ($t = 8.5$, $p < 0.001$). At lower initial resistant allele frequencies, high gene flow (1.43% per day) initially retarded resistance development, but the overall resistance allele frequency was lower in the low gene flow simulations after day 525 (Fig. 2.2b). Resistance evolved fastest when there was a moderate gene flow level (0.143% per day). Fixation of the resistance allele in treated fields occurred only at the low gene flow level, and then only when the initial resistance allele frequency was high. The average resistance allele frequency from day 601 to day 801, measured every 20 days (ca.

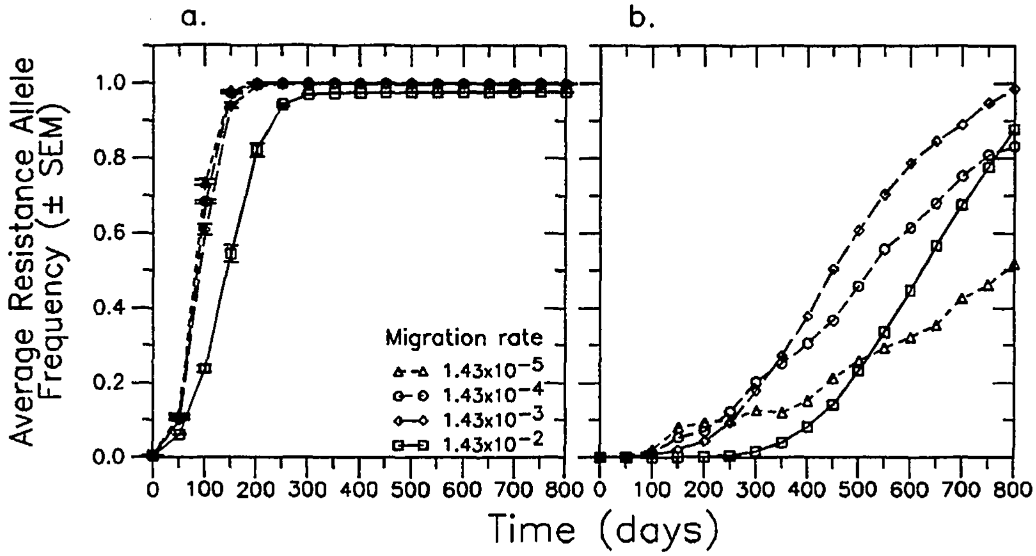

Figure 2.2. Effects of gene flow and initial resistance allele frequency on evolution of resistance among treated fields in a heterogeneous environment (25 fields treated with pesticide, 24 fields untreated). Average resistance allele frequency among all treated fields is shown when the initial resistance allele frequency is: a. - High (0.02); b. - Low (0.0002).

1 generation, Table 2.2), showed a gradual decrease in the proportion of susceptible alleles maintained in the treated fields with decreasing gene flow, rather than a critical value segregating those gene flow levels that might maintain this polymorphism from those levels which would permit local selection and fixation of the resistance allele in treated fields.

Analysis of variance of the time it took 50% of the fields to obtain a resistance allele frequency greater than 50% showed significant gene flow, initial resistance allele frequency, and gene flow x initial resistance allele frequency interaction effects (Table 2.3). The significant interaction term shows that the role of gene flow depended on the amount of genetic variance present initially in the populations.

When resistance was dominant, it evolved more rapidly (Fig. 2.3), but a polymorphism was maintained due to heterozygote advantage in reproductive fitness. The trajectories for the average resistance allele frequencies over time (Fig. 2.3a) indicate that the two higher levels of gene flow approached a different asymptotic value than the lower levels of gene flow. This may be interpreted as a partial switch from local to global selection.

High gene flow rates among heterogeneous fields always retarded resistance evolution among treated fields. Among untreated fields, however, the frequency of the resistance allele was significantly higher in systems with high gene flow. When the dispersal rate was 7.1×10^{-2} per day and resistance was

Table 2.3. Analysis of variance.

	Homogeneous Fields (treated only)				Heterogeneous Fields			
	DF	Mean-Square	F-Ratio	P	DF	Mean-Square	F-Ratio	P
Initial R allele								
Freq. (IRF)	2	2.90	116.2	0.000	2	9.68	213.4	0.000
Gene Flow (GF)	3	0.45	18.1	0.000	3	0.31	6.7	0.002
IRF * GF	6	0.11	4.4	0.004	6	0.13	2.9	0.028
Error	24	0.025			24	0.05		

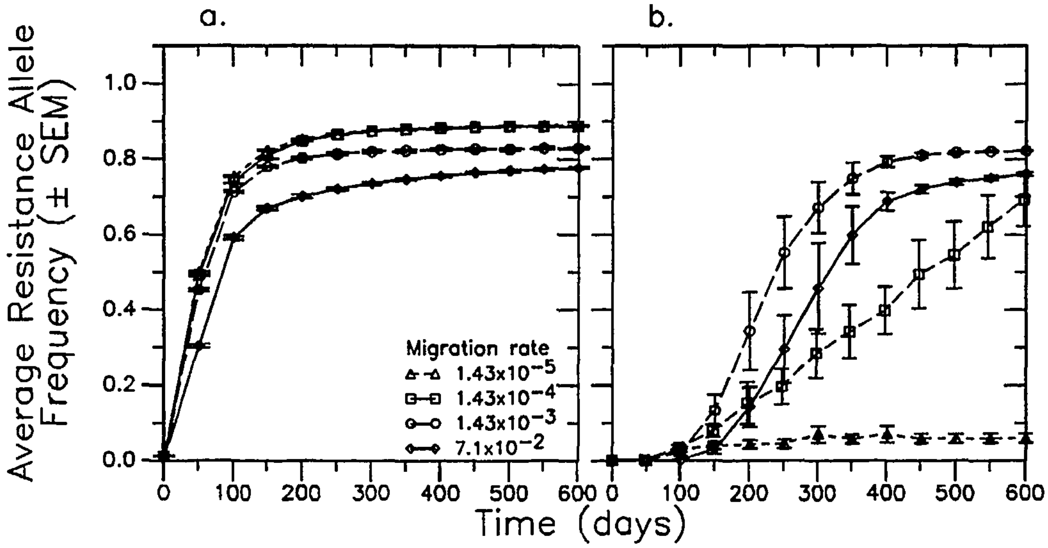

Figure 2.3. Resistance evolution among treated fields in a heterogeneous environment when the resistance allele is dominant. The average resistance allele frequency among treated fields when the initial resistance allele frequency is: a. - High (0.02). b. - Low (0.000005).

dominant, untreated fields had an average resistance allele frequency greater than 50% after 550 days (without ever being treated with insecticide).

Initial Resistance Allele Frequency and Population Size

In the previous simulation, population size was held constant. In these simulations, no migration was allowed between fields, but the population size was varied. The initial resistance allele frequency effect was, however, dependent on population size (Fig. 2.4a). Increased population size resulted in a greater number of alleles in a field, and increased the probability that a field would contain sufficient local genetic variation to adapt without requiring immigration of resistance alleles. The rate of resistance evolution is a constant function of the product of initial resistance allele frequency and population size (the average number of resistance alleles initially in each field, Fig. 2.4b).

Discussion

The simulations presented in this paper demonstrate that the effect of gene flow on local adaptation depends upon the amount of genetic variation initially present in subdivided populations. If some populations lack the full set of genetic variants necessary for adaptation to a novel environment, gene flow may promote rather than retard local adaptation by spreading key genetic material. This effect becomes more pronounced as the adaptive genetic variants decrease in frequency. If resistance alleles are sufficiently rare, occurring in one

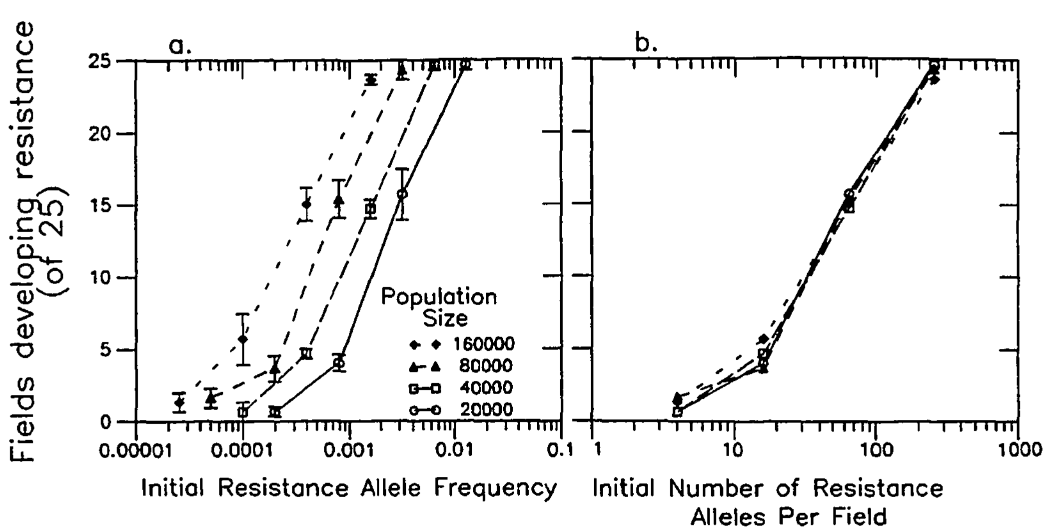

Figure 2.4. Effect of population size and initial resistance allele frequency on evolution of resistance in the absence of gene flow. For each population size, the initial resistance allele frequencies were chosen to hold the initial number of resistance alleles (the initial resistance allele frequency by population size product) constant. The number of fields evolving resistance at: a. - Different initial resistance allele frequencies; b. - Different numbers of initial resistance alleles per field.

or a few fields, resistance evolution in other fields is constrained until gene flow can spread resistance alleles throughout the system or mutation generates them de novo. The overall rate of resistance evolution in this case will be strongly dependent on the rate of gene flow.

These results do not contradict the view that gene flow can retard resistance evolution. When genetic variation is not a constraint, higher levels of gene flow will slow local adaptation in heterogeneous environments by increasing the frequency of maladapted alleles. Simulations show that resistance evolution always occurred more rapidly in the absence of migration out of untreated fields, compared to systems where such gene flow can occur. The effect of gene flow is the net result of interactions between two opposite affects of gene flow: the spread of advantageous rare alleles and the spread of common but maladapted alleles.

The importance of finite populations in considering the effect of gene flow depends on the frequency with which local adaptation is restricted by lack of genetic variation. In the case of insecticide resistance, where initial resistance allele frequency estimates vary from 10^{-3} to 10^{-13} (Roush and McKenzie 1987), resistance evolution may frequently be constrained by the low frequency and high probability of loss of resistance alleles. Our simulations used initial resistance allele frequencies in the upper half of this range (the lowest initial resistance allele frequency was 5×10^{-6}), and the effect of migration speeding local adaptation would only increase as the initial resistance allele frequency was decreased.

Species with small population sizes are also more likely to depend upon gene flow to maintain genetic variance since alleles will tend to become extinct in small populations more rapidly. Rosenheim et al. (1990) found that resistance is less likely to evolve among introduced than native pests. They suggest that resistance evolution is repressed among introduced species by the loss of rare genetic variants during founder events. Their results emphasize that rare alleles limit resistance evolution; the loss of such alleles in finite populations can retard resistance evolution.

Models have demonstrated that high levels of gene flow can retard insecticide resistance evolution (Tabashnik and Croft 1982, Taylor and Georghiou 1979). These models were based on a continent-island model, where gene flow from treated to untreated fields was ignored. Our simulations, which included approximately equal numbers of treated and untreated fields, indicate that high levels of gene flow ($>10\%$ between field types) can retard the evolution of resistance in treated fields, but only at the cost of greatly increasing the resistance allele frequency in untreated fields. We suggest that this occurs as global selection begins to dominate local selection pressures, with the ensuing homogenization of allele frequencies over all field types.

Measurements of gene flow between insect populations have seldom been as high as 10% (Pashley et al. 1985). Measurements of gene flow among diamondback moth populations in Hawaii based on electrophoretic variation (Caprio and Tabashnik, unpublished data) suggest exchange between fields of 5-

10 individuals per generation, which is well below 2% of the individuals in field populations. Gene flow rates at this level are unlikely to significantly retard development of resistance, though they are large enough to facilitate relatively rapid spread of resistance alleles among populations. This is particularly evident in resistance evolution, where there is extremely strong selection for the new phenotype, and treated fields need only be seeded with resistance alleles for resistance to evolve. When selection for the new phenotype is weaker, lower rates of gene flow are likely to retard local adaptation. The movement of rare alleles into new populations will also be delayed, as weak selection pressure for the new phenotype will increase the period during which stochastic influences (and possible loss of alleles) are important. In addition, when selection is weak, more time is required for the frequency of the adaptive allele to increase, a prerequisite for a field to begin to contribute adaptive alleles to the migrant pool.

These results demonstrate that results from models of finite populations can conflict with conclusions based on models of infinite population size. In the latter models, individual subpopulations always contain a complete assemblage of genetic variability. Under natural settings, however, all populations are unlikely to contain all adaptively significant alleles. The speed with which a population can adapt is closely related to the degree to which it can draw on the genetic variability of neighboring populations via gene flow. This conclusion depends on the between-field heterogeneity introduced by random events among finite populations. The extent of differences between results from finite and infinite population models depends on population size, initial genetic variation, and selection intensity. In the case of insecticide resistance evolution, infinite population models had suggested that increases in gene flow retard the rate of resistance evolution. Our finite population models indicate, however, that gene flow is likely to promote evolution of resistance in most cases. Further studies of finite population models are warranted, and may suggest other conditions under which conclusions from infinite population models may not adequately describe evolution in finite populations.

Appendix A

The simulation assumed that demographic entries in the life table for the insect reflect individual probabilities for the event. For example, mortality was the probability that an individual in that stage would die per day. The total number of survivors of a group is the sum of successful Bernouli trials over all individuals in the group. A successful Bernouli trial is a drawing of a random number greater than the mortality rate (Kalos and Whitlock 1986). Thus:

$$N_{f,a+1,g,s} = \mathrm{sum}(\ [r(0,1) > M_{f,a,g}] * N_{f,a,g,s}\ )$$

where:

N = Number in group

M = Mortality rate of group

f = Field

a = Age of group (Given by stage and numbers of days in the stage).

g = Genotype

s = Sex

r(0,1) = A uniform random number between 0 and 1.

$[r(0,1) > M_{f,a,g}]$ evaluates to 1 if true, otherwise 0.

The resultant binomial distribution was simulated using a normal approximation to the binomial with an algorithm given by Kinderman and Ramage (1976). When the expected number of successes or failures was less than 10, a Poisson approximation was used, using an algorithm given by Knuth (1969).

A 32-bit random number generator (Dudewicz et al. 1985) was used for the simulations.

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Chapter 3. A Model for Implementing a Genetically-Improved Strain of the Parasitoid Trioxys pallidus Haliday (Hymenoptera: Aphidiidae)

Abstract

Computer simulation was used to estimate the effects of biological and operational parameters on introduction and establishment of a laboratory-selected strain of the walnut aphid parasite, Trioxys pallidus, resistant to azinphosmethyl. Regional establishment was promoted by migration of the parasite between orchards, low survivorship of the susceptible strain when treated with insecticide relative to the resistant strain, and minimal refuge from insecticide. Splitting available resistant parasitoids into smaller groups and releasing the groups in different orchards increased the speed of implementation. Increasing the frequency of insecticide application or increasing the relative frequency of use of azinphosmethyl over alternative pesticides also accelerated implementation. The model suggests that under current cultural practices in California walnut orchards, establishment of the resistant strain of T. pallidus so that it makes up 50% of the individuals in at least 90% of the orchards will require 5-7 years. Possible genetic improvement projects should be evaluated not only on the potential of genetically improving desirable traits, but also on the potential difficulties of implementing the new strain. Our results suggest that replacement of one natural enemy biotype with another may not always occur in less than five years, despite relatively strong selection for the new biotype. Evaluations to determine the success of

such releases should be planned over an adequate time period, perhaps as suggested by simulation, and not be limited by arbitrary time scales.

Introduction

Genetic improvement uses directed, purposeful genetic alterations to enhance the efficacy of natural enemies for biological pest control (Hoy 1990a). Such genetic alterations may be achieved through artificial selection, hybridization to achieve heterotic effects, or the use of recombinant DNA techniques (Beckendorf and Hoy 1985). Laboratory manipulation can increase the frequency of genotypes that rarely occur in the field. Furthermore, because genetic variability can in some cases be increased considerably beyond that which might ever be obtained under natural conditions, laboratory manipulation has the potential to produce results that might never occur under field conditions.

More than 30 years ago, laboratory selection of parasitoids for resistance to pesticides was recognized as a potentially potent method for enhancing biological control in agriculture. For example, selection with DDT for 19 generations increased resistance 12-fold in the parasite _Macrocentrus ancylivorous_ Rohwer (Hymenoptera: Braconidae) (Pielou 1952, Robertson 1957), but field releases were not reported. The boll weevil parasite _Bracon mellitor_ Say, (Hymenoptera: Braconidae) was also selected for resistance to DDT and toxaphene, but survival of this parasite in pesticide-treated cotton fields was never confirmed (Adams 1967).

Hoyt (1969) showed that the phytoseiid predator, Metaseiulus occidentalis (Nesbitt), which developed resistance to organophosphorus (OP) insecticides in the field, provided effective control of spider mites in Washington apple orchards. Croft and McMurtry (1972) established the OP-resistant strain of M. occidentalis from Washington in southern California apple orchards. Substantial resistance to parathion in the phytoseiid Phytoseilus persimilis Athias-Henriot was achieved with laboratory selection (Schulten and van de Klashorst 1974), but the laboratory-selected strain was not used in the field because a greenhouse-selected strain with greater resistance was found. A laboratory-selected strain of M. occidentalis resistant to carbaryl, OPs, and sulfur has become an effective component of integrated mite management in almond in California (Hoy 1985a). Conservative estimates suggest that this program saves participating growers $24 to $44 per acre per year, which is equivalent to more than $21 million annually for the almond industry (Headley and Hoy 1987).

The parasitoid Aphytis melinus DeBach was selected for resistance to carbaryl in the laboratory (Rosenheim and Hoy 1988) and is being evaluated in citrus groves in California (Spollen and Hoy in preparation). However, pesticide-resistant strains of parasitic Hymenoptera remain rare (Croft and Strickler 1983, Havron 1983, Delorme et al. 1984, Horn and Wadleigh 1988, Hoy 1990b, Tabashnik and Johnson in press, Theiling and Croft 1989).

In commercial walnut orchards in California, Trioxys pallidus Haliday can be an extremely effective parasite of the walnut aphid, Chromaphis juglandicola

Kaltenbach (Schlinger et al. 1960, van den Bosch et al. 1962, van den Bosch et al. 1970, Frazer and van den Bosch 1973, Riedl et al. 1979, van den Bosch et al. 1979). Unfortunately, pesticide applications for the serious pests codling moth, Cydia pomonella (L.), and navel orangeworm, Amyelois transitella Walker, often disrupt biological control of walnut aphid by T. pallidus (UC/IPM 1982). Azinphosmethyl (Guthion) applications have often been associated with secondary outbreaks of walnut aphid (Riedl et al. 1979, Sibbett et al. 1981), yet growers prefer to use azinphosmethyl because it provides more effective control of codling moth and navel orangeworm than alternative pesticides. Screening of field populations and subsequent laboratory selection produced an azinphosmethyl-resistant strain of T. pallidus that also has cross-resistance to other pesticides used in walnut IPM (Hoy and Cave 1988, 1989a, 1989b).

The azinphosmethyl-resistant strain of T. pallidus was reared in the greenhouse on potted walnut trees and released into commercial walnut blocks during the 1988 and 1989 growing seasons. It appeared to establish, survive azinphosmethyl applications, and spread to adjacent treated blocks. Aphid and mummy counts suggested the parasites controlled the aphids in both the azinphosmethyl-treated and adjacent blocks (Hoy et al. 1989, 1990). Results from four release sites suggest that the resistant strain overwintered in low numbers at two sites; survival of parasites at the two other sites appeared poor. Experiments are underway to evaluate the relative fitness of the resistant strain and a susceptible strain under laboratory conditions and to evaluate relative success in overwintering ability. If these experiments indicate that the azinphosmethyl-resistant strain is capable of establishing and overwintering in California walnut orchards, large scale implementation should be considered.

The implementation of genetically-improved organisms can be achieved either through augmentative or inoculative releases. Augmentation requires the ability to rear large numbers of the organism, and there may be little concern for its dispersal capabilities (in fact, it may be preferable to have an organism with limited dispersal capabilities so that it remains in the fields where it is released). The use of an inoculative strategy is more difficult because the new strain must be able to compete well with the native strain while retaining the trait(s) for which it was selected, reproduce and overwinter in the field, and to disperse to, and establish in, new fields. In return, inoculative releases of genetically-improved strains would have lower maintenance costs compared to the augmentative implementation strategy, and could result in a greater benefit:cost ratio for developing this new technology.

Implementing the azinphosmethyl-resistant strain of T. pallidus is a challenge. If the releases are inoculative, this strain must establish, persist in pesticide-treated walnut blocks, overwinter, and successfully compete with the widespread susceptible population. We assumed that the released strain must establish permanently because we did not anticipate that annual mass rearing and releases into individual orchards on a yearly basis would be economically viable.

Because few genetic improvement projects have yet progressed to the field release stage, little is known about the types of biological or operational factors that determine successful implementation. Resistance management models have focused primarily on pests (Taylor 1983, Tabashnik in press), and no previous modeling studies have examined implementation of pesticide-resistant parasitoids. The goals of this project were 1) to assess the potential effects of alternative implementation strategies on the rate of establishment of a laboratory-selected strain of T. pallidus resistant to azinphosmethyl, and 2) to begin to develop criteria for choosing biological control agents for their potential success in genetic improvement programs. We focused on the implementation stage of genetic improvement, i.e., what factors determine the rate at which resistant or otherwise selected populations will replace established populations of natural enemies.

Materials and Methods

Description of Basic Model

The model used for these simulations was a variant of the PopGen model (Chapter 2). This model enabled simultaneous simulation of multiple independent subpopulations that exchange individuals via dispersal, allowing us to examine the effects of spatial heterogeneity and dispersal on resistance evolution among arrays of subpopulations.

Parameters governing individuals of a specific age were given by empirically estimated stage-specific life table parameters such as mortality, reproduction, response to pesticide and propensity to disperse. Each stage (egg/larval, pupal, and two adult stages) was characterized by the number of days the parasitoid spent in the stage, mutation rate, daily migration, and mortality rates. Within each stage, all genotypes were independently characterized by a daily reproductive rate (eggs/day) and a response to pesticide (LC_{50} and slope, derived from probit analysis).

Subpopulations were modelled as finite in size. Dispersal, mortality and other life-table parameters were simulated stochastically. For example, a mortality rate of 0.15 meant that each individual had a 15% chance of dying per day, rather than a deterministic interpretation that 15% of the population would die per day. Each simulation was replicated three times to estimate stochastic variability.

The model assumed that resistance was monogenic, with two alleles at the locus, one of which provided resistance to azinphosmethyl (and some cross-resistance to the alternative pesticides). T. pallidus is a haplo-diploid parasitoid. We assumed that a haploid resistant male (with a single resistance allele) was as resistant as a diploid female homozygous for resistance (Brown et al. in press). Males were therefore either fully susceptible or fully resistant in the simulations.

The simulation model allowed the use of two different pesticides to be incorporated into the model. The two pesticides simulated were azinphosmethyl and a hypothetical alternative pesticide. This alternative pesticide represented the average response of T. pallidus to four other pesticides (Hoy and Cave 1990). Azinphosmethyl treatments decayed with a half-life of 30 days and were ignored after 60 days; the alternative pesticide decayed with a half-life of 15 days and was ignored after 30. These decay rates are based on the response of T. pallidus adults to pesticide residues in leaf-clip bioassays, rather than decay of the actual pesticide, and represent the rate of decay of the parasitoid response to the insecticide. The field application rate used in the model for both azinphosmethyl and the alternative pesticide was 22.9 ppm. This produced mortality rates equivalent to field mortality rates for Guthion 50WP (1 lb. AI), as well as the average field mortality rates of the four other pesticides which comprised our single alternative pesticide (Hoy and Cave 1989b).

Pesticide treatments were applied twice per year, first on a randomly selected day between day 30 and day 45 of the growing season, and a second time between day 70 and day 85. 20% of the fields were treated with azinphosmethyl on the first treatment, while 40% were treated with it on the second treatment. These reflect actual use patterns, where orchards may be treated with insecticide from one to four times per year, with an increasing likelihood of the use of azinphosmethyl later in the season.

Dispersal was modelled as a diffusion process (Rudd and Gandour 1985) characterized by two parameters, the chance that an individual would migrate per day and a diffusion constant which determined the distance that migrants tended to disperse. Most migrants moved to neighboring fields, but there is potential for dispersal to more distant fields.

Density-dependent reduction in adult reproduction occurred via a modified logistic equation:

$$RE = ERE * (1.0 - \sqrt{PS / CC})$$

where:

RE = Reproductive effort

ERE = Expected reproductive effort

PS = Population size

CC = Carrying capacity

Implementation was simulated as a two stage procedure. Stage I simulations examined a single 80-acre orchard divided into 36 2.2 acre blocks in which a release was made into one block. These simulations used a 6x6 matrix of populations with high rates of migration between blocks (30% per generation). Stage II simulations examined between-orchard (regional) events by simulating a 10x10 matrix of 80-acre orchards (8000 acres total) with lower rates of migration between orchards (3% per generation). Stage II simulations began with an initial resistance allele frequency of 15% in the release orchard. The time required for the resistant strain to attain a frequency of 15% in that orchard (from stage I simulations) must be considered when evaluating the total time for implementation.

Biological Parameters for Trioxys pallidus

The life history of T. pallidus was divided into four stages, egg/larval, pupal, and young and old adults (Table 3.1). The reproductive rates for both adult stages depended on the genotype of the female, with a small cost incurred by resistant genotypes (a 5% reduction, varied in sensitivity analysis as described below). The model assumed that T. pallidus was active 200 d/yr, and the remainder of the year was not simulated (a time of 400 d is equivalent to 2 yr). We assumed that 1% of the population in each block or orchard survived the winter and reestablished the subpopulation of T. pallidus the following year.

We did not explicitly model the aphid host. We assumed that the aphid population was sufficient to permit a carrying capacity for the parasitoid, in the absence of pesticide mortality, of $1x10^6$ individuals/block in the stage I simulations (this was varied in sensitivity analysis), and $1x10^7$ individuals/orchard in the stage II full orchard simulations. These correspond to approximately 7500 individuals of all stages per tree in the stage I simulations, and 2100 per tree in the stage II simulations. The model also assumed that aphid populations recovered from pesticide applications more rapidly than the parasitoid.

Parasitoid Response to Pesticide

The response of adult parasitoids to azinphosmethyl and a hypothetical alternative pesticide depended on the parasitoid genotype (Table 3.2). The response of adult parasitoids to the hypothetical pesticide was based on the

Table 3.1. Standard demographic parameters used in the simulation model. Rates are per day. S, wild strain, R, selected strain. Adult1 are adults 1-4 days old, while adult2 are the old stage adults, those 5-8 days old.

Stage	Egg/larva	Pupa	Adult1	Adult2
Days in stage[a]	12	2	4	4
Mortality[a,b]	0.108	0.108	0.125	0.125
Dispersal[c,d]				
Within orchard	0.0	0.0	0.062	0.062
Between orchard	0.0	0.0	0.0062	0.0062
Reproduction (eggs/female/day)[a,e]				
SS	0.0	0.0	50.0	30.0
RS	0.0	0.0	48.75	29.75
RR	0.0	0.0	47.5	28.5

[a] Gutierrez and Nowierski 1979

[b] Frazier and van den Bosch 1973

[c] Sluss 1967

[d] Stary 1988

[e] Schlinger et al. 1960

Table 3.2. Simulated response to pesticides. R = Selected strain, S = Wild strain. Males are haploid and are treated as either RR or SS. Two pesticides were simulated, azinphosmethyl and an alternative pesticide based on the response of T. pallidus to 4 pesticides commonly used in walnut orchards[a]. The response of the immature stages is based on the aphid response to the pesticide. The LC_{50} units are ppm AI, and the initial dose applied when a population was treated with either pesticide was 22.9 ppm.

	SS		RS		RR	
	LC_{50}	slope	LC_{50}	slope	LC_{50}	slope
Egg/larva and Pupal stages						
Azinphosmethyl	16.2	5.59	16.2	5.59	16.2	5.59
Alternative	10.9	2.6	10.9	2.6	10.9	2.6
Adult1 and Adult2 stages						
Azinphosmethyl	5.3	4.05	12.0	4.3	18.66	4.42
Alternative	11.45	4.65	17.97	4.65	22.9	4.65

[a]Data from Hoy and Cave 1989a, 1989b

average response (LC_{50} and slope) of T. pallidus to the pesticides chlorpyrifos, endosulfan, methidathion and phosalone (Hoy and Cave 1989b). Resistance to azinphosmethyl in the resistant strain is partially dominant (Brown et al. submitted), thus the F1 progeny are more like their resistant parents than their susceptible parents (dominance = 0.33; 0 would indicate no dominance). This means that the F1 progeny should survive in the field relatively well compared to the susceptible strain. If the resistance is polygenic as suspected by Brown et al. (submitted), rather than monogenic as assumed here, we expect that establishment may be slower than predicted by our simulations.

The response of the egg/larval and pupal stages to pesticides was based on the response of the aphid to pesticide rather than parasitoid genotype since these life stages depend on the aphid host for survival. Thus, no differential survival of T. pallidus genotypes occurred until the first adult stage. Larvae which survived a treatment within an aphid as a larva/pupa were subjected to pesticide selection when they emerged, though the pesticide had decayed to some degree. There is therefore, differential selection only on newly emerged adults and the adults present in the field at the time of treatment.

Sensitivity Analyses

Sensitivity analysis was performed to determine the stability and sensitivity of the simulation to variation in the following parameters: initial frequency of the resistant phenotype, total population size, selection intensity (response of the wild-type genotype to the pesticides), refugia size, relative fitness, and dispersal rates. Unless noted, all sensitivity analysis was done within simulated 80-acre orchards with releases made into a single block (stage I).

Initial Frequency. The initial frequencies of the resistant phenotype in the release block were 30, 60 (the default), and 90%. Pretreatment of actual release blocks in the field with azinphosmethyl prior to release was expected to lower the susceptible parasitoid population and result in a high initial release frequency in release blocks.

Population Size. The carrying capacity in T. pallidus individuals per block was varied from $5x10^4$, $1x10^5$, $1x10^6$ (the default) to $1x10^7$. The population size includes all individuals in all four life stages.

Selection Intensity. The differential survival or selection intensity of the susceptible vs. selected strains was varied by altering the survivorship of the susceptible strain when treated with azinphosmethyl. Simulations were completed with a survivorship of the susceptible strain at the field application rate of azinphosmethyl of 0.1%, 0.5% (=default), 1%, and 10%, while the survivorship of the resistant strain was held constant at 33% for all simulations.

Refuge Size. In walnut orchards, large portions of individual trees may not be fully treated with pesticide (though incomplete coverage may be offset by high rates of within block dispersal by the parasitoid). The values for refugia, the proportion of the population not treated with pesticide, under sensitivity analysis were 1, 5, 10 (the default), and 30%.

Relative Fitness. The relative fitness of homozygous resistant females was altered by reducing fecundity. Fecundity in these individuals was reduced by 2.5, 5, 10 and 20% compared with susceptible homozygotes. The fecundity of female heterozygotes was reduced by half the amount of resistant homozygotes.

Dispersal Within Orchards (Stage I). The rates of dispersal between blocks by adults (the percentage of the adult population which migrates per generation) examined for sensitivity analysis were 1, 5, 10 and 30% (the default). The diffusion constant was 0.35, which resulted in 89.4% of the migrants moving to neighboring orchards, 10.6% moving two orchards away and 0.1% moving three orchards.

Dispersal Rates Between Orchards (Stage II). Because there is little empirical evidence on between-field dispersal rates, we also used sensitivity analysis to examine how fast the resistant strain was established in the large scale model (8000 acres, 100 orchards) at four different migration rates. The rates examined were (per generation): 30% (the default for the within orchard simulations), 10%, 3% (the default for the large scale simulations), and 1%.

Implementation Strategies. The first implementation strategy was examined within an 80-acre orchard (stage I). All other strategies were tested under stage II (regional scale) simulations.

Strategy 1. Effect of Number of Release Sites Within an 80-Acre Orchard. We compared the effects of release of all the resistant parasitoids in one block (initial resistance allele frequency of 60% in that block) with release of the same number of resistant parasitoids split among four blocks (each with an initial resistance allele frequency of 15%).

Strategy 2. Effect of Susceptibles on the Rate of Establishment of the Resistant Strain. To determine how rapidly the resistant strain would establish in the absence of established susceptible populations, a set of simulations was run using standard parameters with the exception that all fields were empty except for the released resistant individuals. These simulations can be compared to stage II simulations with releases made into one orchard to determine the effect of established susceptible populations on the rate of establishment of the resistant strain.

Strategy 3. Effect of Number of Release Sites in 8000 Acres. We examined the effects of releasing a fixed total of resistant parasitoids into either 1, 4, 16, or 32 orchards.

Strategy 4. Effect of Pesticide Use Patterns. We varied both the frequency with which orchards were treated, and the proportion of orchards that were treated with azinphosmethyl rather than the alternative pesticide. The frequency of pesticide treatment was increased by adding a third application prior to day 130, with 60% of the orchards treated with azinphosmethyl (the other two sprays were left at 20 and 40% azinphosmethyl, respectively). In a second set of simulations, the proportion of fields on which azinphosmethyl was used was increased from 20 and 40% for the two sprays to 80% for both (the number of sprays applied remained the same). Use of azinphosmethyl increased selection for the resistant strain because this pesticide decayed slower and the ratio of survival of the resistant strain to the susceptible strain was higher than when the alternative pesticide was used. Finally, we also examined the case in which three applications were simulated per year, with 80% of the orchards being treated with azinphosmethyl.

We emphasize that we are not advocating specific pesticide use or specific cultural practices, as such decisions must be made in a larger context of pest management. These simulations were designed to determine the effects such alterations might have on the implementation of the resistant T. pallidus strain.

Statistics

We used two approaches to analyze the time-series data (resistance allele frequencies over time) produced by the simulations. To analyze the sensitivity analysis data, we compared the average allele frequencies for both release and non-release blocks on the final day of the experiment using ANOVA. We also measured the rate at which the resistance allele was established by determining the amount of time it took for 90% of the non-release orchards to have a resistance allele frequency greater than 50%. These data, also analyzed by ANOVA, were more appropriate for comparing rates of establishment for the resistant strain.

For data analysis, we assumed that the initial resistance allele frequency was equal to the proportion of resistant individuals released into the population.

Random mating resulted in Hardy-Weinberg equilibrium between the susceptible and resistance alleles by the following (second) generation.

Results and Discussion

Sensitivity Analyses

Initial frequency. The initial resistance allele frequency in the release plot had relatively little effect on the final frequency of the resistance allele in the 35 non-release blocks in a single orchard (Fig. 3.1a, Table 3.3). Changing the initial frequency of resistant parasitoids from 30% to 90% resulted in an 11.5% increase in the resistance allele frequency in the non-release blocks after three years (from 71% to 83%). Although these differences were statistically significant ($F=13.2$; $df = 2,6$; $P=0.006$), they should have little effect operationally. When resistant parasitoids were released at a high frequency (90%), the difference between the concentration of resistance alleles in the emigrant and immigrant populations to the release plot was greatest. Most of the susceptible immigrants were replacing resistant emigrants, and the initially high concentration of resistant parasitoids was rapidly diluted. This dilution caused the resistance allele frequency in the release plot to drop throughout the first year, from 90 to 36.3%, despite selection for that allele with two applications of azinphosmethyl (Fig. 3.1b). In contrast, when resistant parasitoids were released at low frequencies (30%), dilution

Table 3.3. Results of sensitivity analyses. ANOVA were conducted using arcsine transformed data. A block is an area equivalent to 1/36 of an 80-acre orchard. Data represent the mean resistance allele frequency over fields of three replicates after 3 years.

Parameter Varied	Release Field		Nonrelease Fields	
	Mean	SD	Mean	SD
Initial frequency of resistant strain in release block				
30%	0.77^{A}	0.01	0.72^{A}	0.02
60%	0.85^{B}	0.02	0.81^{B}	0.02
90%	0.84^{B}	0.02	0.83^{B}	0.01
	$F_{(2,6)}=13.2$ $p=0.006$		$F_{(2,6)}=57.7$ $p<0.001$	
Block (within orchard) carrying capacity				
50,000	0.84^{A}	0.05	0.82^{A}	0.03
100,000	0.81^{A}	0.03	0.78^{A}	0.05
1,000,000	0.82^{A}	0.03	0.78^{A}	0.05
10,000,000	0.79^{A}	0.06	0.76^{A}	0.07
	$F_{(3,8)}=0.642$ $p=0.609$		$F_{(3,8)}=0.916$ $p=0.475$	
Refuge size				
1%	0.92^{A}	0.02	0.89^{A}	0.02
5%	0.89^{A}	0.02	0.86^{A}	0.02
10%	0.82^{B}	0.03	0.78^{B}	0.04
30%	0.53^{C}	0.01	0.47^{C}	0.01
	$F_{(3,8)}=181.7$ $p<0.001$		$F_{(3,8)}=136.5$ $p<0.001$	
Selection intensity (Mortality of susceptible homozygotes at field dose)				
0.1%	0.87^{A}	0.008	0.84^{A}	0.01
0.5%	0.78^{B}	0.04	0.74^{B}	0.05
1%	0.77^{B}	0.03	0.74^{B}	0.03
10%	0.36^{C}	0.02	0.34^{C}	0.01
	$F_{(3,8)}=198.7$ $p<0.001$		$F_{(3,8)}=149.1$ $p<0.001$	

Means within the same column and parameter subheading followed by the same letter are not significantly different (P = 0.05); Duncan's [1955] multiple range test.

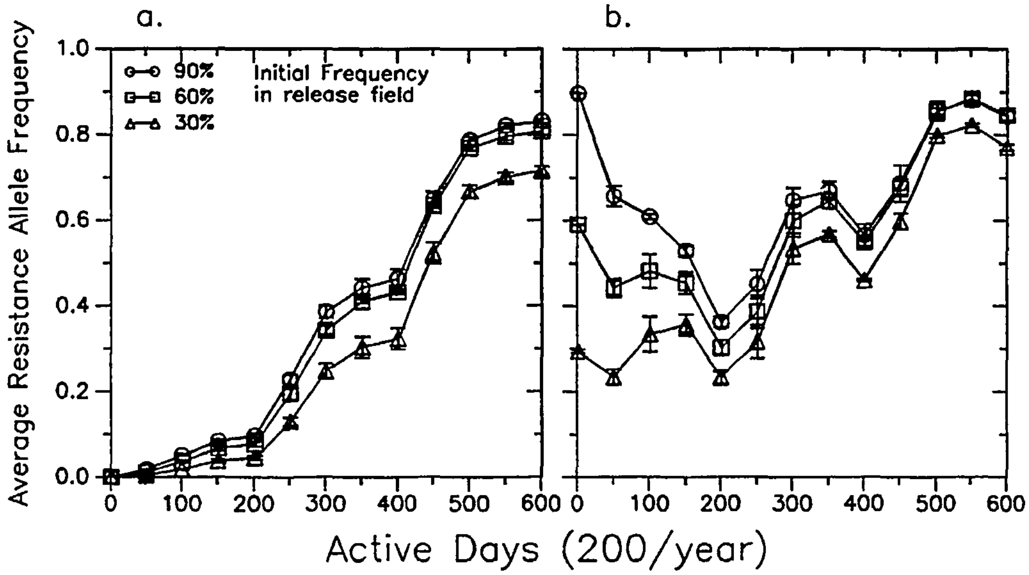

Figure 3.1. Sensitivity of simulation results to changes in the initial resistance allele frequency in the single release block. a. Non-release blocks. b. Release block.

by susceptibles occurred more slowly. Only 30% of the emigrants were likely to be resistant individuals, and most of the susceptible immigrants into the release plot were therefore replacing susceptible emigrants. The resistance allele frequency in these plots dropped from 30 to 23.3%. The difference in resistance allele frequency in the release blocks between the high and low releases dropped from 60% initially to 12.5% at the end of the first year.

Population Size. The maximum population size for each plot did not significantly affect the average resistance allele frequency after three years in either the non-release (Fig. 3.2a, Table 3.3) or release plots (Fig. 3.2b, Table 3.3). We had expected that larger populations would produce more migrants and thus increase establishment rates of resistant T. pallidus in non-release plots. The absence of this effect suggests that migration rates were high enough so that all plots were inoculated rapidly even when population sizes were low. An alternative explanation is that individuals with resistance alleles that have just moved into a new plot constitute a smaller initial frequency in the new population when the population size is large. The increased numbers of migrants at a larger population size would therefore be offset by the longer time required for selection to increase the resistance allele frequency. In other words, a given number of migrants add less genetic variance upon which selection can act when moving to a large population than to one which is smaller.

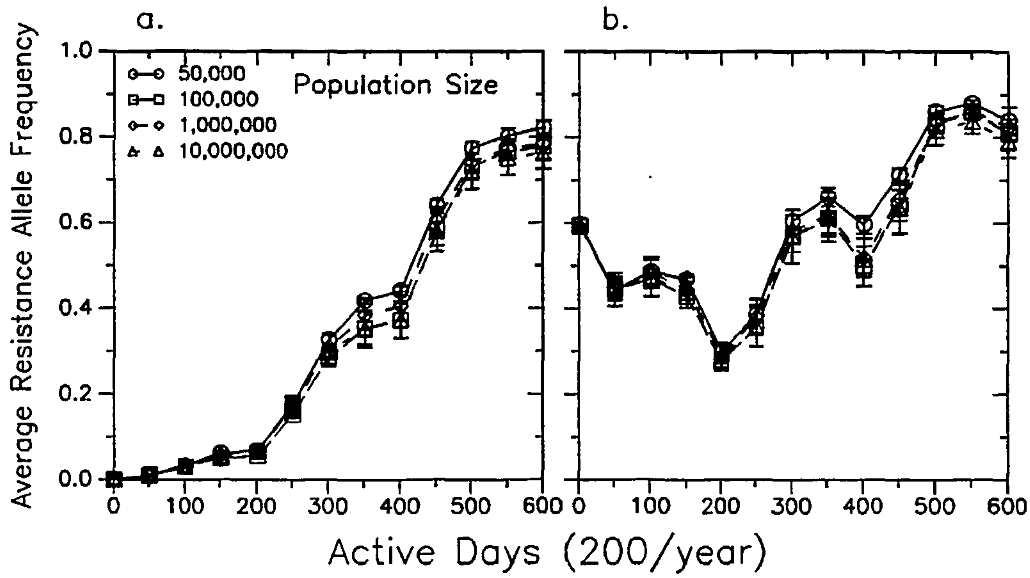

Figure 3.2. Sensitivity of simulation results to changes in the carrying capacity of each block in an orchard (1 block = 2.2 acres). a. Non-release blocks. b. Release block.

Selection Intensity. Variation in the survival of susceptible T. pallidus following an azinphosmethyl treatment from 1% to 0.1% had relatively little effect on the average resistance allele frequency in non-release plots after three years (Fig. 3.3a, Table 3.3). Increasing the survival of susceptible T. pallidus to 10% (compared with 33% for the resistant strain) after an application of azinphosmethyl, however, greatly slowed penetration of the resistance allele into susceptible populations. After three years, the average resistance allele frequency among non-release plots was 34% if survival of susceptibles was 10%, while it had risen to 73.7% when only 0.5% of the susceptibles survived azinphosmethyl applications. Variations in selection intensity had similar affects in the release plots (Fig. 3.3b, Table 3.3). Thus, the results indicate that the outcome of the simulations are unlikely to be substantially different unless there are greater than 5-fold changes in this parameter.

Refuge Size. Results showed that reducing the refuge size to 10% markedly increased the rate of establishment, but further reductions had little effect (Fig. 3.4a, Table 3.3). Thus, extensive efforts to decrease the refuge size below 5-10% are probably not warranted, as they will have little impact on the rate of establishment of the resistant strain. If the actual value of this parameter is much larger than 10%, our standard assumption, our simulation results may overestimate the speed with which resistant populations become established.

Relative Fitness. Increasing the fitness reductions associated with the resistant strain decreased the average resistance allele frequency among non-release fields after three years (Fig. 3.6a, Table 3.4). Within the range examined, the decrease in the resistance allele frequency was linearly related to the fitness reduction (Fig. 3.7a, r^2=.994).

Dispersal Within Orchards (Stage I). Increasing the rate of dispersal increased the rate at which the resistant allele established in the non-release fields (Table 3.4). As with selection intensity and refuge size, the sensitivity of the simulations to variation in the migration rate was nonlinear. Small changes in the migration rate when the rate was low caused large differences in the results, while similar changes at higher rates caused much less change in the results (Fig. 3.7b, Table 3.4). Our standard parameter value for migration (10%) lies within a relatively stable area. The nonlinearity of the sensitivity analysis further suggests that increasing the migration rate will not cause large changes in establishment rates, but that similar changes in the opposite direction (lowering the migration rate to <10%/generation) can significantly retard the replacement of susceptible populations by the resistant strain.

Dispersal Rates Between Orchards (Stage II). Higher rates of dispersal caused faster spread of resistant parasitoids (Table 3.4), and the time required for 90% of the fields to have a resistance allele frequency greater than 50% varied significantly among migration rates (Kruskal-Wallis test = 9.97, P=0.019, df = 3;

Table 3.4. Results of sensitivity analyses. ANOVA conducted using arcsine transformed data. All data represent mean resistance allele frequency over fields of three replicates after three years except for the stage II dispersal simulations, where the mean is taken after five years.

Parameter Varied	Release Field		Nonrelease Fields	
	Mean	SD	Mean	SD
Fitness reduction in resistant strain				
2.5%	0.84^{A}	0.03	0.81^{A}	0.01
5%	0.82^{A}	0.03	0.78^{A}	0.02
10%	0.74^{B}	0.04	0.69^{B}	0.04
20%	0.54^{C}	0.06	0.47^{C}	0.06
	$F_{(3,8)}=30.2$ $p<0.001$		$F_{(3,8)}=43.2$ $p<0.001$	
Dispersal between blocks (stage 1, within orchard simulations)				
1%	0.98^{A}	0.002	0.38^{A}	0.03
5%	0.95^{AB}	0.02	0.65^{B}	0.08
10%	0.91^{BC}	0.04	0.75^{BC}	0.06
20%	0.85^{C}	0.04	0.77^{C}	0.03
30%	0.84^{C}	0.06	0.82^{C}	0.07
	$F_{(4,10)}=11.43$ $p<0.001$		$F_{(4,10)}=24.3$ $p<0.001$	
Dispersal between orchards (stage 2, regional simulations)				
1%	0.997^{A}	0.002	0.72	0.002
3%	0.99^{B}	0.001	0.85	0.033
10%	0.99^{C}	0.002	0.92	0.009
30%	0.95^{D}	0.006	0.94	0.011
	$F_{(3,8)}=116.3$ $p<0.001$		Kruskal-Wallis test=9.974 $p=0.019$ df=3	

Means within the same column and parameter subheading followed by the same letter are not significantly different (P = 0.05); Duncan's [1955] multiple range test.

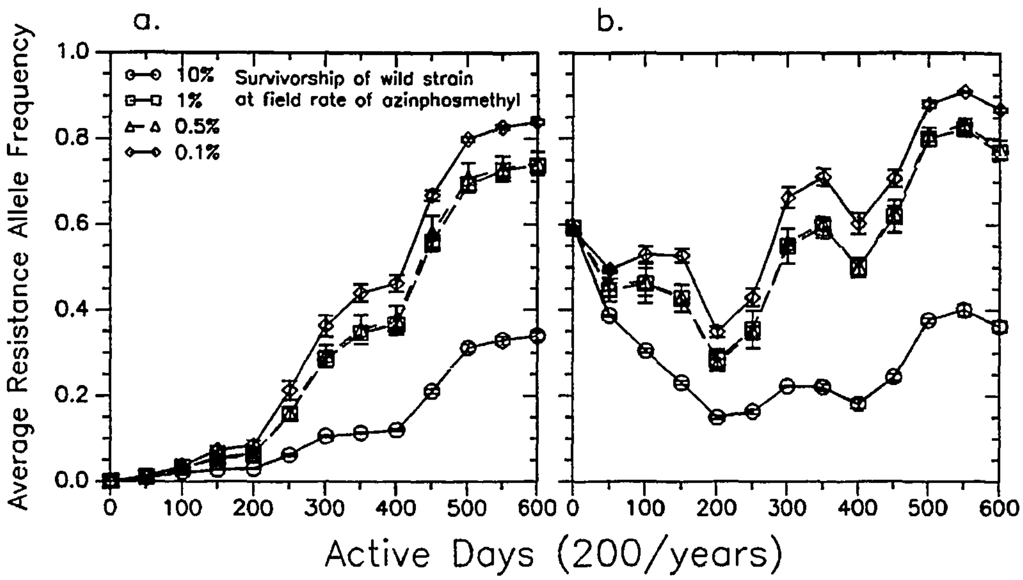

Figure 3.3. Sensitivity of simulation results to changes in selection intensity between the resistant and susceptible strains. a. Non-release blocks. b. Release block.

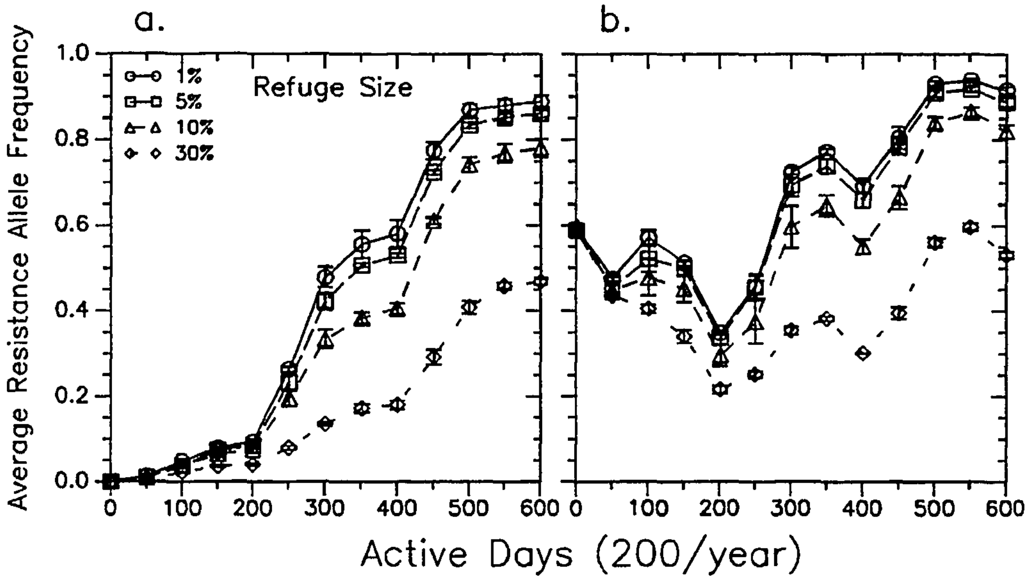

Figure 3.4. Sensitivity of simulation results to changes in refugium size (the percent of the population which is unaffected by an insecticide application). a. Non-release blocks. b. Release block.

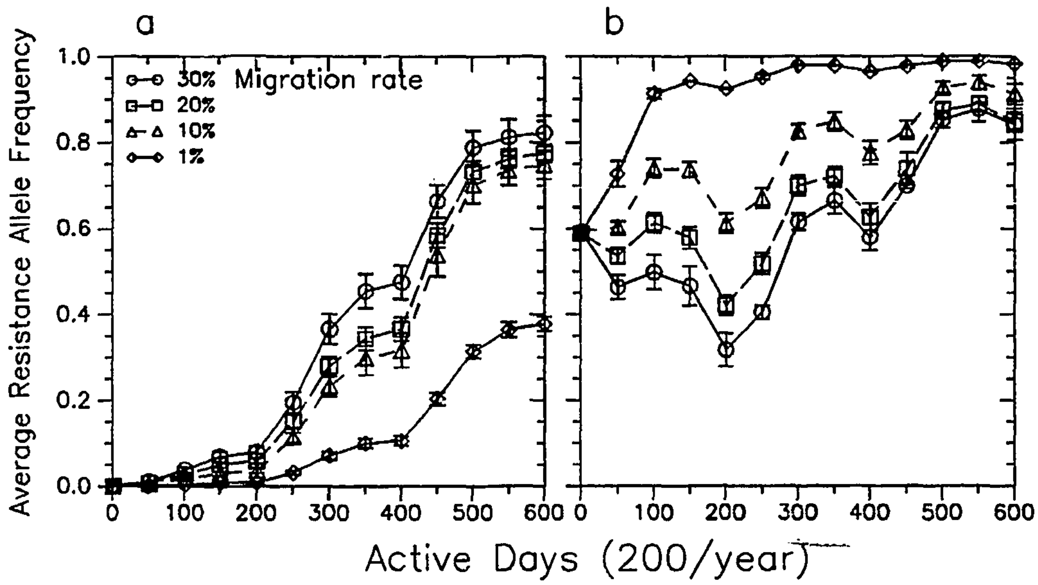

Figure 3.5. Sensitivity of simulation results to changes in between block (within orchard) migration rates. The migration rate is the percent of the population which migrates per generation. a. Non-release blocks. b. Release block.

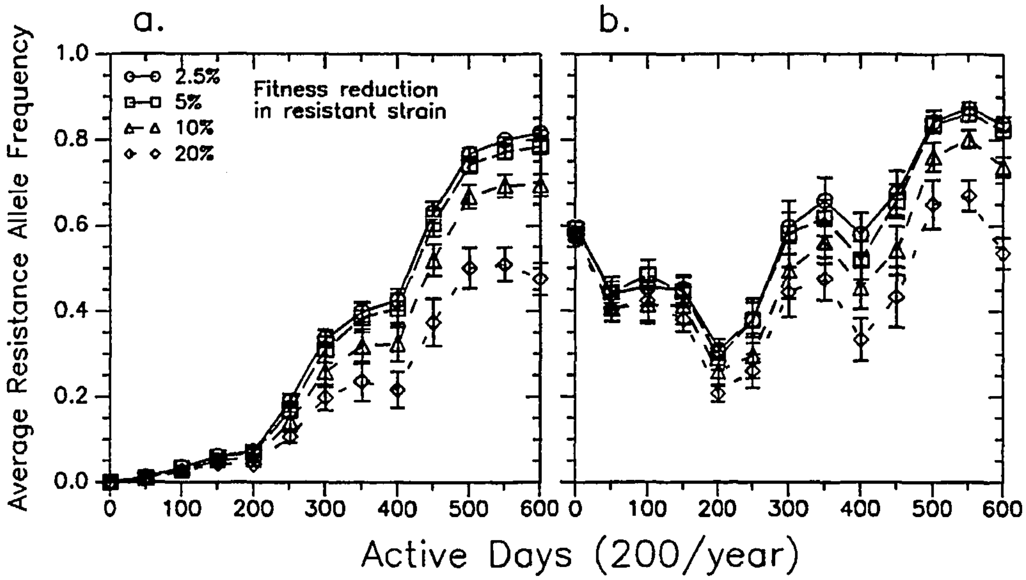

Figure 3.6. Sensitivity of simulation results to changes in the relative fitness of the resistant strain. Fitness was reduced by decreasing the fecundity of resistant females. a. Non-release blocks. b. Release block.

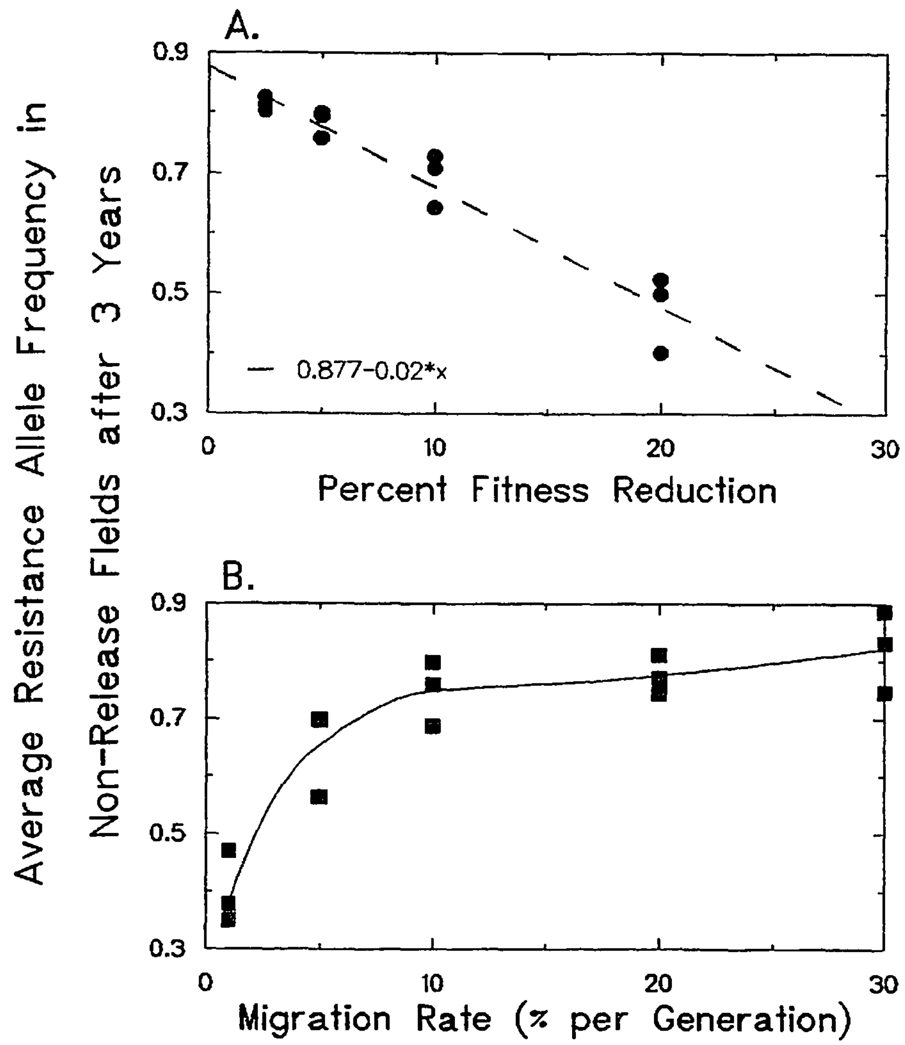

Figure 3.7. Sensitivity of simulation results to variations in two parameters. a. Fitness decrease in a resistant homozygote compared with a susceptible homozygote in an untreated environment. The response is a linear function of the fitness reduction. b. Percent migration per generation. The response is a non-linear function of the migration rate (it is more sensitive to changes at lower migration rates).

Wilkinson 1988). However, establishment of the resistant biotype never occurred in less than three years, and operationally the differences between establishment in four vs. five years is not of major importance.

Implementation Strategies

Strategy I. Effect of Number of Release Sites Within an 80-Acre Orchard. The number of release sites within an orchard (1 versus 4) did not significantly affect resistance allele frequencies after three years in either the release ($t = 0.032$, $P = 0.976$, $df = 4$) or non-release blocks ($t = 0.04$, $P = 0.970$, $df = 4$). Mean resistance allele frequency after three years in the non-release blocks was 71.3% when all resistant parasitoids were released in one block, compared with 71.4% when the same number of parasitoids were split up and released at four different foci. These results suggest that the number of release sites within an orchard is not an important factor in establishing a resistant population in an orchard, at least for a highly mobile insect such as T. pallidus. Single releases within each release orchard may limit labor and distribution costs.

Strategy 2. Effect of Susceptibles on the Rate of Establishment of the Resistant Strain. Previous establishment of susceptible populations of T. pallidus significantly slowed the rate of establishment of resistant T. pallidus in one hundred 80-acre walnut orchards. In the absence of susceptible T. pallidus, resistant populations were established in all 100 orchards within one year from the time of the initial release (Fig. 3.8). The rapid spread of resistant T. pallidus in the absence of already-established susceptible populations is consistent with the observed spread of T. pallidus when originally released in the San Joaquin Valley (van den Bosch et al. 1962, 1970). The data suggest that release of the resistant strain of T. pallidus in areas where a susceptible strain does not already exist would result in rapid establishment.

On the other hand, when a resistant population was released but susceptible populations were already present, almost six years were required before the resistance allele frequency in 90% of the non-release orchards exceeded 50%. Establishment in the original release orchard of a resistance allele frequency of 15% required 1.25 years (based on stage I simulations, the stage II simulations assumed that the initial release field had already established a resistance allele frequency of 15%), and it took an additional 4.5 years for the resistance allele to spread and establish in the other 99 orchards (Table 3.5, 3% migration).

The long time required for the replacement of the original susceptible biotype with a new resistant biotype was unexpected given that the survival of the resistant biotype was 66 times greater after an azinphosmethyl treatment and 6.1 times greater following the alternative pesticide treatment than the susceptible biotype (Hoy and Cave 1988, 1989). The simulations suggest several factors which contribute to the difficulty of establishing the resistant biotype including refuge size, high reproductive capacity, and relatively low selection intensity for the resistant strain (due to operational factors such as frequency of use, and type

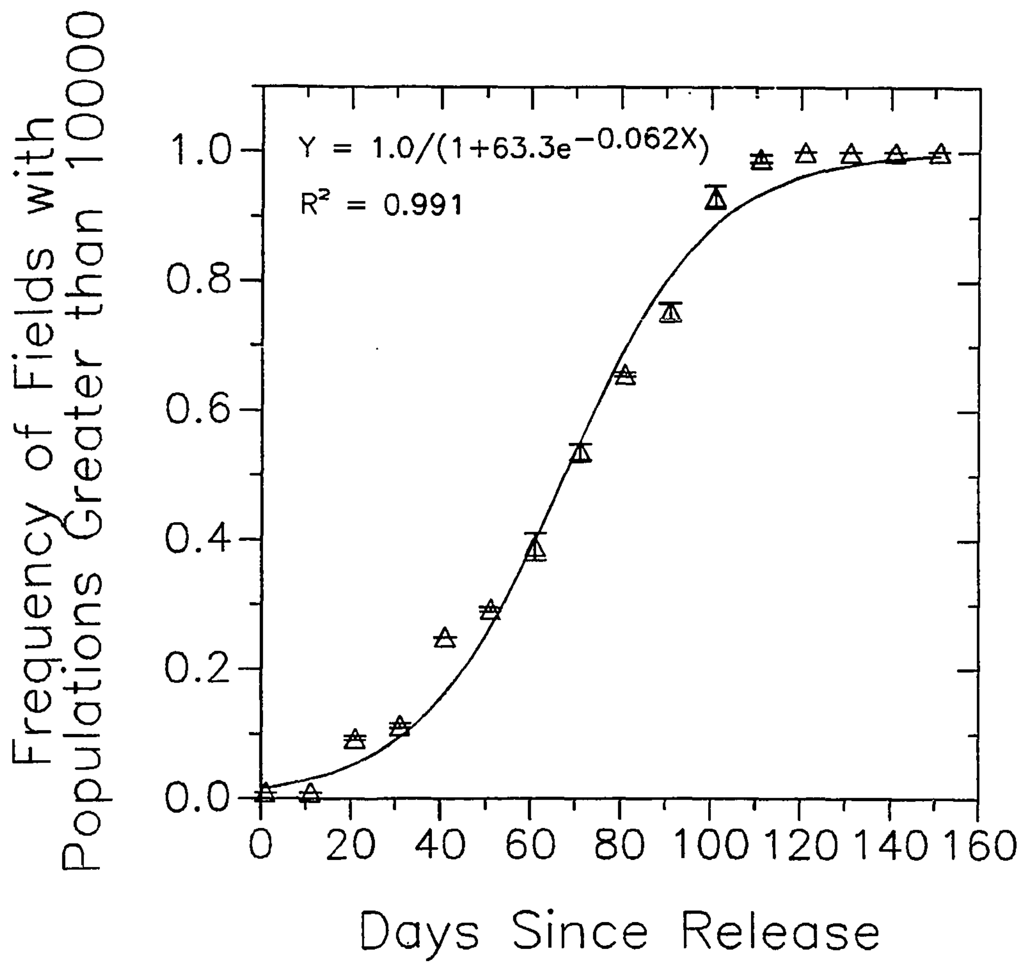

Figure 3.8. Proportion of orchards with established T. pallidus populations (out of 100), when there were no previously established T. pallidus populations. These were single stage simulations and the time shown is the entire time required for implementation.

Table 3.5. Time required (days ± S.D.), assuming *T. pallidus* is active 200 days/year) for 90% of the orchards in a 100 orchard simulation to have a resistance allele frequency greater than 50% at different migration rates. It is assumed that the original release orchard had attained a resistance allele frequency of 15% at the start of these simulations (which required approx. 1.25 years in the within orchard simulations).

	Migration rate (%/generation)			
	1	3	10	30
Release field				
	91 (10)	101 (-)	111 (10.0)	424 (80.8)
Non-release fields				
	1041 (-)	927.7 (72.3)	761 (45.8)	667.8 (20.8)

of pesticide used). Refuge size is important because even low survivorship rates translate into large absolute numbers; for example, 0.1% survival of a population consisting of 10,000,000 individuals still leaves 10,000 following selection. With a refuge size of 10%, 110,000 would remain following selection. This example also suggests that refuge size and selection intensity may interact, i.e., with a large refugium, increasing selection intensity so that only 1000 individuals survive selection would probably have little effect on the outcome of the simulations because so many more individuals (100,000) could survive in refugia. A second important factor was the high reproductive rate of the parasitoid, which allowed susceptible populations to recover more rapidly after pesticide treatments and return towards the carrying capacity of the aphid population in the walnut orchard. Migrants moving into recently treated orchards with lower population sizes would have a proportionally larger effect on the allele frequencies in that population than migrants that move into the field after the population had recovered. High reproductive rates therefore reduce the time during which a population is recovering from a pesticide application and within which immigrants would benefit from continued population expansion.

A third factor was that the selection for the resistant strain was not as great as expected a priori because of relatively infrequent pesticide applications (twice per year), and the frequent use of pesticides other than azinphosmethyl that decayed rapidly and selected less severely for the resistant strain. Selection between genotypes in the model is also reduced because the immature stages of

T. pallidus are assumed to respond to pesticide selection based on their host genotype. There is no net selection for any genotype until the parasitoids emerge as adults. Any mortality prior to emergence affects all genotypes the same. Though selection for resistant phenotypes still occurs when the parasitoids emerge, the pesticide residues will have decayed and the selection intensity will have decreased from the time of the initial spray application.

Strategy 3. Effect of Number of Release Sites in 8000 Acres. The rate of establishment, measured as the average resistance allele frequency over all fields (rather than the non-release fields, since the 32 release fields constitute a major portion of all the fields), increased as resistant parasitoids were divided into smaller groups and released into more fields (Fig. 3.9). The rate of increase, however, decreased with the number of release foci, suggesting that there is an optimum number of release foci which is dependent on the economics associated with the cost of release per focus and the expected increase in rate of establishment. In contrast to these results, the number of release foci (1 or 4) did not have a significant effect on the rate of establishment in within-orchard simulations. In the within-orchard simulations, there were higher migration rates which rapidly moved the resistance allele to most fields, alleviating the need to artificially spread the allele by releasing at more foci.

Strategy 4. Effect of Pesticide Use Patterns. Increasing the number of sprays per year or increasing the proportion of azinphosmethyl use for each spray increased the establishment rate of the resistance allele (Table 3.6). The most

Table 3.6. Time required (days ± S.D., assuming T. pallidus is active 200 days/year) for 90% of the non-release orchards to have a resistance allele frequency greater than 50% with different implementation strategies. Means followed by different letters are significantly different at the 5% level (Duncan's (1955) multiple range test).

# sprays/year	% of orchards to which azinphosmethyl was applied	Time (SD)
2	20, 40	927.7 (72.3)A
2	80, 80	661.0 (17.3)B
3	20, 40, 60	691.0 (10.0)B
3	80, 80, 80	497.7 (11.5)C

ANOVA, $F_{3,8} = 106.5$, $p<0.01$ (analysis performed on log transformed data)

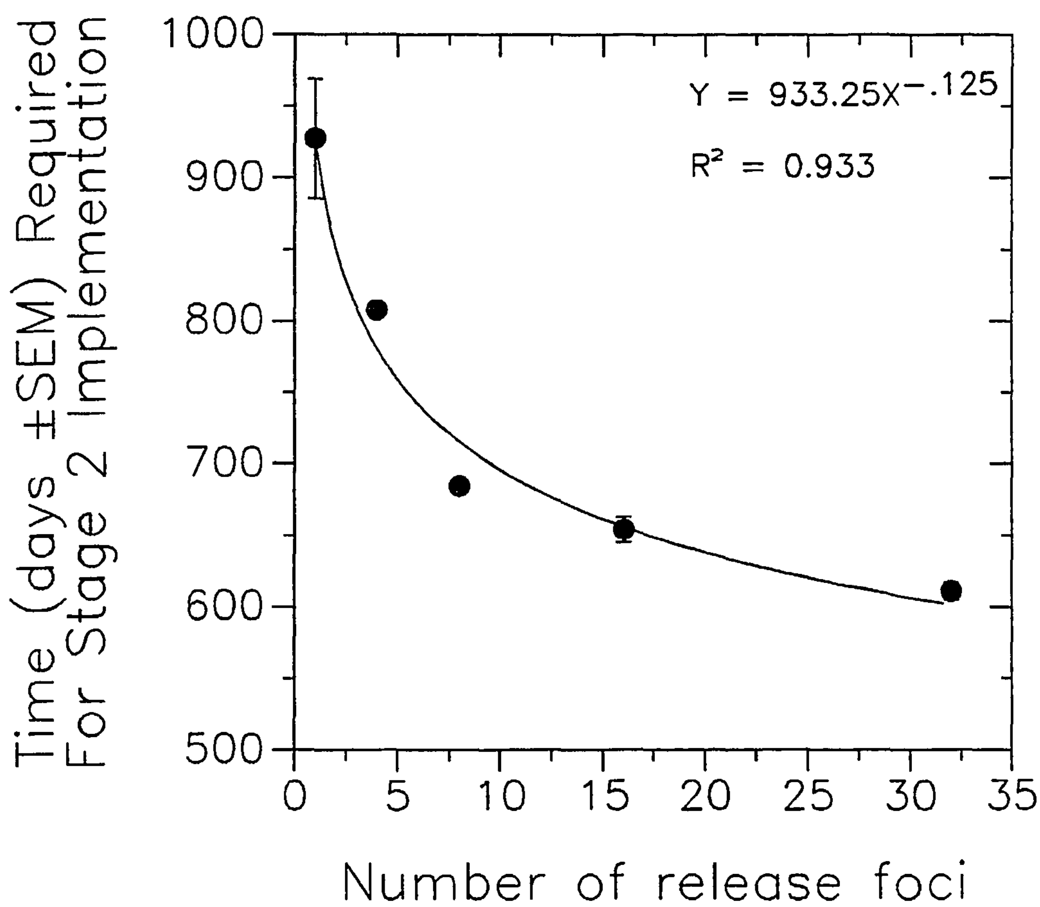

Figure 3.9. Time required for the resistance allele to have a frequency of more than 50% in at least 90% of the orchards when the same number of parasitoids were divided up and released at an increased number of foci. The R^2 value is based on transformed data (log(time) = log(A) + B * log(no. foci)).

rapid implementation occurred when three sprays per year were applied with 80% of the applications using azinphosmethyl. Implementation still required approximately 3.5 years in this optimistic scenario (1 year to establish the resistance allele to 15% in the release orchard, and 2.5 years to establish it in the remaining 99 non-release orchards).

Conclusions

Our simulations suggest that the laboratory-selected strain of T. pallidus will take 5-7 years to become established in California walnut-growing areas unless multiple releases are made or cultural practices are changed. Several factors contribute to the difficulty of establishing this laboratory-selected, pesticide-resistant strain. Most important is the presence of an already established susceptible strain. Despite selection pressures for the azinphosmethyl-resistant strain, the high reproductive rate of surviving susceptible parasitoid populations limit the potential for highly effective migration and establishment of the resistant strain in new orchards to relatively brief periods following pesticide applications when population levels have been decreased. At other times, resident susceptible populations close to the carrying capacity limit the reproductive success of resistant immigrants. A migrant moving into an expanding population (because the population was treated) contributes more to the allele frequencies of the new population than if it enters the population at carrying capacity when its expected reproductive success would be ~1 (replacement). While we do not know how often field populations of T. pallidus are limited by aphid densities, we do know that: 1) at some times of the year, large proportions of aphids (20-80%) collected from the field are parasitized (Frazier and van den Bosch 1973, Hoy et al. 1989, 1990, Nowierski 1979, van den Bosch et al. 1962), and 2) T. pallidus populations will often track walnut aphid populations, suggesting that the aphid populations at low levels limit reproductive success of T. pallidus. Nowierski (1979) found a strong correlation ($r^2=0.926$) between aphid and T. pallidus densities and suggested a weak delayed density-dependent parasite response. These observations suggest that the realized reproductive success of T. pallidus in the field may be limited by aphid densities.

A second problem in implementing the resistant strain of T. pallidus is the difficulty involved in rearing large numbers for release, thus reducing the number of fields which can be inoculated with the resistant strain. Rearing currently requires growing walnut trees in pots in the greenhouse, rearing pure colonies of walnut aphids in cages, preventing contamination of aphid colonies by other parasites, predators, or diseases, and synchronizing exposure of aphid hosts so T. pallidus can parasitize appropriate-sized hosts. These operations are all labor intensive and costly (Cave and Hoy, in preparation). If it were possible to release resistant parasitoids into most orchards, the resistant strain could be established in most fields within 2-3 years (based on the results from the stage I simulations).

Relatively large refugia (estimated to be 10%) caused by the difficulty in adequately treating large walnut trees also slow replacement of the susceptible strain, though sensitivity analysis suggests that decreasing the refugium size to 1% may not increase the rate at which the resistant strain replaces the susceptible by more than 10-15%. The opposite, though, is not true, and increasing the refuge size to 30% could increase the time required for establishment of the resistant biotype in most orchards by 50%.

The ability of the resistant strain to become established is also affected by selection frequency and intensity. Increasing the relative survival of the resistant versus susceptible T. pallidus 5-fold in the sensitivity analysis had little effect on the overall penetration of the resistance allele. Increasing the frequency of treatment applications, so that selection occurs during more of the season, had a larger effect on the rate of establishment of the new biotype. This may also be achieved by increasing the proportion of azinphosmethyl used in applications which are normally made, as this pesticide has a longer residual activity than the alternative pesticides (Hoy and Cave 1989a, 1989b). More frequent treatment applications would also keep resident susceptible population levels lower so that resistant migrants to an orchard would have greater reproductive success.

The model suggests that higher rates of migration will speed up implementation. This is particularly true when the density of migrants (a product of both migration rate and population size) is low. In the case of T. pallidus, our simulations suggest that when migration is very low (<10% per generation), long delays may occur before orchards most distant from the release orchard receive resistant migrants, during which time selection cannot act in those orchards due to lack of variation. At higher rates of migration, there is a greater chance that migrants from the original release orchard will inoculate distant orchards. Further increases in migration rates only increase the number of migrants to these orchards. Though this increases the genetic variation and increases the establishment rate of the resistance allele, the response is not as dramatic as increases at low migration rates, where selection was hindered by the lack of variation, rather than the amount of variation. As expected, increasing the number of release foci (artificially increasing the migration rate) in the stage II simulations (where there was a low migration rate) had a larger effect on the establishment rate than increasing the number of release foci in the stage I simulations.

The results of these simulations indicate that there are a number of criteria which can be used to suggest whether or not a parasitoid might be a good candidate for a genetic improvement program when an inoculative release with permanent establishment is anticipated. These criteria are:

1. Ease of Rearing. When large natural populations of the parasitoid already exist, it is essential to be able to rear large numbers of the new biotype if establishment is expected to be successful over large areas in a short period of time.

2. Relative Survival of Resistant and Susceptible Genotypes and Treatment Frequency. The more often the parasitoid is treated, the more sensitive it is to pesticides and the longer the residual activity of the pesticide, the easier it will be to replace a susceptible biotype with a resistant one. Parasitoids in agroecosystems which are treated more frequently may be more readily replaced by a new resistant biotype than parasitoids in less intensively treated systems. It also might be preferable, from the implementation standpoint, to choose a species with a lower natural tolerance to pesticides for a genetic improvement project, because it may be easier to develop a strain in which the difference in survival of the resistant and susceptible genotypes is greatest. This suggestion must be tempered by the fact that the naturally less tolerant species may have less genetic variability with which to select for resistance. This also implies that parasitoids which do poorly (i.e., are rare) in pesticide treated fields because of poor tolerance, but are otherwise efficient biological control agents, might actually be good candidates for screening for genetic improvement. Those parasitoids which are common are exactly those which will be hardest to replace (because of high tolerance and large population sizes).

3. Additional Factors. Other factors that affect how rapidly a new biotype can be implemented include migration rate, refuge size and dominance of expression of the trait differentiating the biotypes. The parasitoid should have a migration rate which exceeds some critical value (our simulations suggest $>1\%$ per generation in the case of T. pallidus) so that it can spread to new fields

rapidly. Very high gene flow, however, may also retard the implementation, and an intermediate rate of gene flow is probably optimal (Takahata 1983, Chapter 2). The presence of large unselected populations in refugia can significantly retard the establishment of the resistant biotype. Refugia in the form of untreated fields are less likely to affect the rate of establishment than are refugia due to poor coverage within treated fields. Untreated fields, while they are unselected and are likely to remain susceptible longer, can affect selection for resistance alleles in treated fields only to the degree to which migrants can move between the fields. In contrast, susceptible parasitoids which survive in a refugium within a treated field are already "in place". Such refugia, however, are affected by selection (the individuals in the refuge are not selected, but they are drawn from a population which was selected), and the proportion of susceptible parasitoids within these refugia will decline more rapidly than in isolated untreated fields.

While not examined by our simulations, the dominance of the resistance trait has been demonstrated by many models (Comins 1977, Taylor and Georghiou 1979, Tabashnik and Croft 1982) to have an important effect on the rate at which the resistant biotype is established. When the resistance allele first moves into a new field, it will be present at a low frequency and exist predominantly in heterozygotes. Increasing the expression of the resistance allele in the heterozygotes will result in greater selection and faster establishment of the resistant strain.

The conclusions of this paper are not limited to the release of genetically-improved biotypes resistant to pesticides, but may be important in other biological control programs where the release of a new biotype is being considered. While the specific form of selection may be different, i.e., mate competition, heat tolerance, and so on, many of the same factors (dispersal rates, selection intensity, population size, etc.) will be important in determining whether the new biotype establishes and the time required to displace the previously established biotype.

The use of computer simulation to examine potential implementation strategies of a laboratory-reared parasitoid strain resistant to pesticides has allowed us to organize a large body of biological data collected on T. pallidus and the walnut aphid (including field life-table studies, response to pesticides, and movement), and to incorporate them into a unified predictive tool. While further validation of the model may require refinement of the parameters, the general trends and predictions of the simulations, based on the sensitivity analysis, are unlikely to be radically different. The model has proven useful in evaluating possible implementation strategies for the resistant strain of T. pallidus, and may provide guidelines for implementation of future genetically altered strains. It suggests that releases of new biotypes of biological control agents in regions with already established strains of the species may require substantial time to replace the original strain. Under such conditions, evaluation efforts may need to extend beyond 3-5 years before the ultimate success of the release can be assessed.

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