

locus, regenerating a 10-kb Sal I fragment of the *CHL1* gene, the same size found in wild-type plants (Fig. 3A). Sequence analysis of the *CHL1* gene in four of the revertants verified that the element had excised, leaving behind a small insertion (Fig. 2). In addition, new restriction fragments that hybridized with radiolabeled *Tag1* sequences were evident in the revertants (Fig. 3B). Thus, in the revertants, *Tag1* or *Tag1*-related elements had inserted into new loci. We conclude that *Tag1* is a mobile transposable element.

To confirm that *Tag1* is an endogenous element of *Arabidopsis*, genomic DNA was isolated from the untransformed parent used to construct the transgenic *Ac* lines. The parent originated from the ecotype Landsberg and carries the morphological mutation *erecta*. Southern blot analysis with radiolabeled *Tag1* DNA indicated that the Landsberg *erecta* parent contains *Tag1* and two additional *Tag1*-related elements, each present in only one copy per haploid genome (Fig. 4). No *Tag1* or related sequences were found in two other ecotypes of *Arabidopsis*, Columbia and Wassilewskija (Fig. 4).

By selecting for chlorate-resistant mutants of *Arabidopsis* from a population carrying an active *Ac* element, we have trapped a new mobile *Arabidopsis* transposon. *Tag1* transposition may have been stimulated in the Landsberg plants by the DNA breakage or genomic stress caused by the integration of T-DNA into the *Arabidopsis* genome, by the transposition of *Ac* (13), or by the propagation of the plant cells in tissue culture (14). Upon activation, the element transposed to the *chl1* locus and, when homozygous, produced *chl1* mutant progeny. We think it unlikely that the *Ac* transposase directly mobilizes *Tag1*, as no *Ac* transposase binding site (AAACGG) is found adjacent to the inverted repeats of *Tag1* as it is in *Ac* (15). Whatever the mechanism of activation, the now mobile *Tag1* should be useful for tagging plant genes.

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## Soil Quality and Financial Performance of Biodynamic and Conventional Farms in New Zealand

John P. Reganold,\* Alan S. Palmer, James C. Lockhart, A. Neil Macgregor

Biodynamic farming practices and systems show promise in mitigating some of the detrimental effects of chemical-dependent, conventional agriculture on the environment. The physical, biological, and chemical soil properties and economic profitability of adjacent, commercial biodynamic and conventional farms (16 total) in New Zealand were compared. The biodynamic farms in the study had better soil quality than the neighboring conventional farms and were just as financially viable on a per hectare basis.

Concerns about environmental, economic, and social impacts of chemical or conventional agriculture have led many farmers and consumers to seek alternative practices that will make agriculture more sustainable. Both organic and biodynamic farmers use no synthetic chemical fertilizers or pesticides, use compost additions and manures to improve soil quality, control pests naturally, rotate crops, and diversify crops and livestock. Unlike organic farmers, biodynamic farmers add eight specific preparations, made from cow manure, silica, and various plants, to enhance soil quality and plant life (1).

We examined soil properties and financial performance on pairs or sets of biodynamic and conventional systems over a 4-year period (1987 to 1991) on the North Island of New Zealand (Table 1). We also

made financial comparisons between these farms and representative conventional farms in each study region on the basis of models used by the New Zealand Ministry of Agriculture and Fisheries (MAF) (2). A farm pair consisted of two side-by-side farms, one biodynamic and one conventional; a farm set consisted of three adjacent farms, one biodynamic and two conventional. The choice of five farm pairs and two farm sets (totaling 16 farms) was made on the basis of surveys, interviews, and on-farm soil examinations of more than 60 farms to ensure that all soil-forming factors, except management (3), were the same in each farm pair or set.

The biodynamic farms had been managed biodynamically for at least 8 years, with the oldest for 18 years, to provide time for the biodynamic farming practices to influence soil properties. The farm pairs or sets included a range of representative farming enterprises in New Zealand: market garden (vegetables), pip fruit (apples and pears), citrus, grain, livestock (sheep and beef), and dairy. Farms in each pair or set had the same crop and livestock enterprise. Paddocks (fields) chosen for study in each

J. P. Reganold, Department of Crop and Soil Sciences, Washington State University, Pullman, WA 99164.  
A. S. Palmer and A. N. Macgregor, Department of Soil Science, Massey University, Palmerston North, New Zealand.  
J. C. Lockhart, Department of Agricultural and Horticultural Systems Management, Massey University, Palmerston North, New Zealand.

\*To whom correspondence should be addressed.

farm pair or set had soils in a single soil profile class and were located at the juncture of adjoining farms. The soil of each paddock was sampled at numerous locations (4). In total, 130 soil samples from 22 paddocks were taken and analyzed (5).

In six of the seven farm sets (Table 2), the biodynamically farmed soils had better structure and broke down more readily to a good seedbed than did the conventionally farmed soils. The crumb and nut structures found predominantly on the biodynamic farms provide better aeration and drainage for crop or grass growth compared with the blocky and clod structures found mostly on the conventional farms (6). Soil was more friable, which makes it more easily tilled by

farm machinery, on four of the seven biodynamic farms compared with that of their conventional neighbors.

The surface soil bulk density was significantly less on four of the biodynamic farms than on their conventional counterparts (Table 2); when all data were aggregated, bulk density was significantly lower on the biodynamic farms (Table 3). Bulk density is related to mechanical impedance and soil structure, both of which affect root growth. Penetration or cone resistance is another indicator of mechanical impedance. Two of the three biodynamic farms in pasture had significantly lower penetration resistances in the upper 20 cm than their conventional counterparts had. The results were variable

for the horticultural and mixed farms (Table 2). Overall (Table 3), the biodynamic farms had a significantly lower penetration resistance in the upper 20 cm; there was no difference between farming systems in soil 20 to 40 cm below the surface.

Organic matter content, soil respiration, mineralizable nitrogen, and the ratio of mineralizable nitrogen to organic carbon were significantly higher on almost all the biodynamically farmed soils than on the conventionally farmed soils (Table 2). The aggregated data (Table 3) indicate significantly higher values for these four parameters on the biodynamic farms. The higher amounts of organic matter on the biodynamic farms have contributed to better soil

**Table 1.** General farm characteristics. Abbreviations: bio, biodynamic; veg, vegetables; con, conventional; pip, pip fruit; cit, citrus; and org, organic.

Farm	Main enterprise	Number of years (1966 to 1991)	Farm size (ha)	Paddocks* per farm	Fertilizer† (1983 to 1991)	Pesticides and pest management (1983 to 1991)
Bio veg	Market garden	13 con; 4 org; 8 bio	11	1	Manures, composts, bonemeal, fishwastes, biodynamic preparations	Cultural controls,‡ biological controls,§ copper and sulfur sprays
Con veg	Market garden	25 con	45	1	12-5-14 and 12-10-10 of N-P-K	Propyzamide, alachlor, maneb, propineb, vinclozolin, methamidophos
Bio pip	Pip fruit	10 con; 15 bio	5	2	Composts, fish manures, biodynamic preparations	Cultural controls‡
Con pip 1	Pip fruit	25 con	7	1	12-10-10 of N-P-K, potassium superphosphate	Terbacil, simazine, glyphosate, chloropyrifos, guthion, azocyclotin, polyram, captan, triadimefon, dodine, bitertanol
Con pip 2	Pip fruit	25 con	24	1	12-10-10 of N-P-K	Amitrole, simazine, terbacil, glyphosate, guthion, azocyclotin, chloropyrifos, polyram, captan, dodine, bitertanol, myclobutanil, fruit-fed ANA
Bio cit	Citrus	17 con; 8 bio	10	3	Composts, fish fertilizer, biodynamic preparations	Copper spray,   biological controls§
Con cit 1	Citrus	25 con	12	2	Nitrphoska (N-P-K fertilizer), urea, superphosphate with trace elements	Glyphosate, paraquat, acephate, copper oxychloride
Con cit 2	Citrus	25 con	9	1	Urea, superphosphate, fertigation with ammonium nitrate, calcium nitrate, sulfate of potash	Glyphosate, terbutylazine plus terbumeton, dimethoate, clofentezine, thiazolidone, diazinon, copper oxychloride, maneb plus zinc and manganese, benornyl
Bio mixed	Grain, sheep, and beef	15 con; 10 bio	202	1	Rock phosphate, seaweed, composts, biodynamic preparations	Cultural controls,‡ biological controls§
Con mixed	Grain, sheep, and beef	25 con	280	1	Superphosphate, urea, chlormequat chloride¶	MCPA + triazine, MCPB 2,4-D, chlorsulfuron, pirimicarb, terbuconazole, cultural controls‡
Bio livestock	Sheep and beef	12 con; 13 bio	180	1	Fish fertilizer, biodynamic preparations	Cultural controls,‡ biological controls§
Con livestock	Sheep and beef	25 con	445	1	Fish fertilizer, rock phosphate, chicken manure	MCPA, glyphosate, picloram, dimethyl carbate
Bio dairy 1	Dairy	1 con; 6 org; 18 bio	25	1	Rock phosphate, seaweed, fish fertilizer, biodynamic preparations	Cultural controls,‡ biological controls§
Con dairy 1	Dairy	25 con	51	1	Potassium superphosphate	MCPA
Bio dairy 2	Dairy	15 con; 10 bio	235	2	Biodynamic preparations	Cultural controls,‡ biological controls§
Con dairy 2	Dairy	25 con	150	2	15-10-10-8 of N-P-K-S, urea	2,4-D

\*Number of paddocks or fields where soil was sampled on a particular farm. †Includes organic and synthetic chemical fertilizers. ‡Cultural pest controls include physical and mechanical practices such as rotating and diversifying crops, green manuring, clearing weeds from field borders, and altering the timing or way of planting. §Biological pest controls involve the introduction or buildup of natural predators, parasites, and pathogens that keep pest populations below injurious numbers. ||Approved chemical spray by the New Zealand biodynamic and organic certification boards. ¶A plant growth regulator.

**Table 2.** Mean values of analyzed soil properties (5). Soil samples were taken at a depth of 0 to 10 cm, except where noted. Data from those farm pairs in which each farm had only one paddock were analyzed with the use of standard *t* tests (12). Data from farm pairs or sets

with a total of four or six paddocks (that is, pip fruit, citrus, and dairy 2) were analyzed with analysis of variance (ANOVA) so that the variation due to different enterprises or soils was removed (13).

Soil property	Farm identification															
	Bio veg	Con veg	Bio pip	Con pip	Bio cit	Con cit	1, 2	Con 1, 2	Bio mixed	Con mixed	Bio livestock	Con livestock	Bio dairy 1	Con dairy 1	Bio dairy 2	Con dairy 2
Texture	Silty clay loam	Silty clay loam	Silty clay loam	Silty clay loam	Silty clay loam	Silty clay loam	Silty clay loam	Silty clay loam	Silty clay loam	Silty clay loam	Sandy loam	Sandy loam	Clay loam	Clay loam	Fine sandy loam	Fine sandy loam
Structure	Nut blocky	Clods, blocky	Nut, blocky	Clods, blocky, massive	Nut, granular	Nut, granular	Nut, blocky, granular	Nut, blocky, granular	Clods, nut	Nut, crumb	Nut, blocky	Nut, blocky	Blocky, nut	Blocky, nut	Nut, crumb	Nut, blocky
Consistence	Friable	Firm, friable	Friable	Firm	Friable	Friable	Friable, firm	Friable, firm	Friable	Friable	Friable	Friable	Firm	Firm	Friable	Firm, friable
Bulk density (0 to 5 cm) (Mg m <sup>-3</sup> )	1.10	1.22*	1.06	1.30†	1.00	1.00	1.04	1.08	1.01	1.18	1.16	1.12*	1.12*	1.12*	1.13	1.24†
Penetration resistance (0 to 20 cm) (MPa)	1.50†	1.31	1.92	2.66*	2.96	2.96	3.02	2.29†	1.87	2.64	3.38†	3.38†	4.61	4.61	3.45	4.41†
Penetration resistance (20 to 40 cm) (MPa)	2.63*	2.24	2.25	2.66†	3.92†	3.92†	3.24	3.19†	2.75	3.47	3.92*	3.92*	4.96	4.96	4.07	4.76†
Carbon (%)	4.30†	3.06	4.79†	3.85	6.48†	6.48†	5.58	4.37	5.50†	3.83†	2.99	2.99	5.32	5.32	4.13†	3.45
Respiration (μl O <sub>2</sub> hour <sup>-1</sup> g <sup>-1</sup> )	54.6†	12.6	49.2*	39.0	96.7†	96.7†	60.2	42.0†	32.1	89.0	87.6	87.6	71.9	71.9	70.3†	51.4
Mineralizable N (mg kg <sup>-1</sup> )	112.6†	62.6	141.1†	111.9	150.6†	150.6†	106.4	87.5*	65.7	71.1†	51.8	51.8	191.3	191.3	194.8*	153.7
Ratio of mineralizable N to C (mg g <sup>-1</sup> )	2.61†	2.01	2.99	2.96	2.42†	2.42†	1.98	2.04†	1.20	1.92	1.76	1.76	3.63	3.63	4.71	4.45
Topsoil thickness (cm)‡	—§	—	23.0†	20.0	19.7	19.7	17.9	21.7	19.5	22.8	17.3	17.3	17.8	17.8	28.0	27.6
CEC [cmol (+) kg <sup>-1</sup> ]	24.4†	16.9	30.3	28.5	23.9	23.9	22.4	22.8	26.0	13.8†	7.8	7.8	17.8*	17.8*	21.9*	18.8
Total N (mg kg <sup>-1</sup> )	4200†	2690	4020	3490	6120†	6120†	5060	4680	6140*	3940†	2950	2950	5780	5780	4590†	4110
Total P (mg kg <sup>-1</sup> )	3840†	2220	1210	990	2320	2320	2860†	1390	2060*	590†	380	380	1610†	1610†	1200	1350†
Extractable P (mg kg <sup>-1</sup> )	157.0*	124.7	67.6	56.8	65.9	65.9	132.0†	11.5	16.4†	11.7†	7.5	7.5	62.3†	62.3†	22.2	45.5†
Extractable S (mg kg <sup>-1</sup> )	5.7	17.8†	11.2	10.2	11.3	11.3	56.3†	37.3	28.4	3.8	2.4	2.4	8.5†	8.5†	5.8	7.1
Extractable Ca (cmol kg <sup>-1</sup> )	17.7†	12.8	21.4	23.3	16.8	16.8	16.0	9.3	13.4†	5.1	5.3	5.3	12.4†	12.4†	12.4	11.1
Extractable Mg (cmol kg <sup>-1</sup> )	1.76†	0.54	3.59†	3.04	1.54	1.54	2.23†	1.16	1.23	0.74	0.83	0.83	0.90	0.90	2.02†	1.73
Extractable K (cmol kg <sup>-1</sup> )	1.25*	0.84	2.80†	1.87	0.60	0.60	1.18†	1.54	1.92	0.12	0.22†	0.22†	0.31	0.31	1.04	0.75
pH	6.84*	6.70	6.40	6.73*	6.21	6.21	6.30	5.72	5.72	5.94	6.47†	6.47†	5.78*	5.78*	6.06	6.07

\**P* < 0.05. †*P* < 0.01. ‡Topsoil thickness includes surface and subsurface (A) horizons. §Topsoil made. ||Cation exchange capacity in centimoles of cation charge (+) per kilogram of soil. could not be measured on the conventional farm because of deep tillage operations, so no comparison was charge of specified cation per kilogram of soil.

**Table 3.** Mean values of aggregated soils data. Data were analyzed with ANOVA so that the variation due to different enterprises or soils was absorbed or removed (13).

Soil property	All bio farms	All con farms
Bulk density (Mg m <sup>-3</sup> )	1.07	1.15*
Penetration resistance (0 to 20 cm) (MPa)	2.84	3.18*
Penetration resistance (20 to 40 cm) (MPa)	3.55	3.52
Carbon (%)	4.84*	4.27
Respiration ( $\mu\text{l O}_2$ hour <sup>-1</sup> g <sup>-1</sup> )	73.7*	55.4
Mineralizable N (mg kg <sup>-1</sup> )	140.0*	105.9
Ratio of mineralizable N to C (mg g <sup>-1</sup> )	2.99*	2.59
Topsoil thickness (cm)†	22.8*	20.6
CEC (cmol kg <sup>-1</sup> )‡	21.5*	19.6
Total N (mg kg <sup>-1</sup> )	4840*	4260
Total P (mg kg <sup>-1</sup> )	1560	1640
Extractable P (mg kg <sup>-1</sup> )	45.7	66.2*
Extractable S (mg kg <sup>-1</sup> )	10.5	21.5*
Extractable Ca (cmol kg <sup>-1</sup> )§	12.8	13.5
Extractable Mg (cmol kg <sup>-1</sup> )§	1.71	1.68
Extractable K (cmol kg <sup>-1</sup> )§	0.97	1.00
pH	6.10	6.29*

\* $P < 0.01$ . †Topsoil thickness includes surface and subsurface (A) horizons. ‡Cation exchange capacity in centimoles of cation charge (+) per kilogram of soil. §Centimole charge of specified cation per kilogram of soil.

structure and consistence and to bulk density and cone resistance that are lower than those of their conventional neighbors. Soil respiration and the ratio of mineralizable nitrogen to organic carbon give an indication of the microbial activity of the soil, which accounts for the recycling of vital nutrients such as nitrogen, phosphorus, and sulfur for plant growth (7).

Earthworms were counted on the two market gardens to give another indication of biological activity. From 30 soil cores (15 cm in diameter by 15 cm deep) taken on each paddock, we found the biodynamically farmed soil to average 175 earthworms per square meter compared with 21 earthworms per square meter on the conventionally farmed soil. By mass, the biodynamically farmed soil had 86.3 g of earthworms per square meter, whereas the conventionally farmed soil had 3.4 g of earthworms per square meter. These differences were most likely due to the use of pesticides, shown to reduce earthworm populations (8), on the conventional farm.

Topsoil was significantly thicker on two biodynamic farms than on their conventional neighbors (Table 2). Overall, 2.2 cm more topsoil was present on the biodynamic farms (Table 3). These differences were

partly due to the significantly lower soil bulk densities on the biodynamic farms. Greater organic matter content and biological activity contributed to the formation of topsoil at a faster rate on the biodynamic farms. Soil erosion was not significant on any of the paddocks in this study.

Cation exchange capacity and total nitrogen were more often higher on the individual biodynamic farms, whereas total and available phosphorus, available sulfur, and soil pH were more often higher on the individual conventional farms (Table 2). This relation, except for total phosphorus, holds true when the aggregated nutrient data were compared (Table 3). Aggregated amounts of calcium, magnesium, and potassium were similar in the two systems. There were a number of statistically significant differences in the amounts of phosphorus, sulfur, potassium, calcium, and magnesium between individual farms, although few differences were of biological significance (that is, almost all soils were of adequate fertility for their respective crops) (9).

To evaluate financial viability, we examined farmers' annual accounts from 1987 to 1991. These accounts are the only common source of farm financial data in New Zealand because few New Zealand farmers keep financial records of individual farm enterprises beyond annual accounts (10). Reliable economic data from annual accounts were available for 11 of the 16 farms. We compared the financial performance of the biodynamic farms both with that of their conventional neighbors and with that of the average, representative conventional farm (2) in the region of each farm pair or set. Most of the products from the biodynamic farms were sold as certified organic or biodynamic at a premium price up to 25% higher than the market price of a similar conventional product.

Profits can be different from one farm to another because of the ownership structure or the amount of fixed costs such as debt servicing. To compensate for these differences, we excluded fixed costs from our calculations and used an analysis of enterprise gross margins as a measure of financial performance (11). Gross margin is the difference between total farm income per hectare and variable or operating expenses per hectare. Examples of variable costs include those of fertilizers, pesticides, biodynamic preparations, fuel, and labor. We only examined farming enterprises requiring similar commitments of owner-operator resources per hectare, except for dairy farm pair 2, where the biodynamic farm was selling yogurt and the conventional farm milk. Here, the additional direct costs of yogurt production were included in the gross margin analysis of the biodynamic farm.

One biodynamic farm (livestock) had greater, two biodynamic farms (mixed and dairy 2) had lower, and two biodynamic farms (market gardens and citrus) had similar gross margins compared to those of their conventional neighbors (Table 4). Compared with the representative conventional farms (2) in their regions, three biodynamic farms (citrus, livestock, and dairy 1) and three conventional farms (mixed, livestock, and dairy 2) were more prosperous, two biodynamic (mixed and dairy 2) were less prosperous, and one conventional farm (citrus) was comparable. In the majority of cases, the biodynamic farms had less year-to-year variability in gross margin than did the conventional farms. Economic stability is one of the most significant characteristics of sustainable farming systems. Total income and variable costs were not consistently lower or higher on the biodynamic farms than on their adjacent conventional neighbors or the MAF representative (2) conventional farms.

From farmer interviews and their annual accounts, we determined that the biodynamic citrus, livestock, and dairy 1 farms have been able to secure reliable markets for their products, which is an important factor for economic stability. Gross margins for the biodynamic market garden were less than for the conventional counterpart in 1988 and 1989 but greater in 1990 and 1991. Annual returns per hectare for the biodynamic market garden have increased consistently over this 4-year period because of the development of biodynamic or organic markets and improved productivity and farm management practices. The biodynamic mixed farm (except in 1991) and the biodynamic dairy farm 2 have not matched the annual gross margins representative of conventional farms in the same region.

Although gross margins provide a comparison of financial performance of two farms under different management approaches, total gross margins illustrate the financial return to each whole farm or to the major farm enterprise. Total gross margin is simply the gross margin times the effective enterprise area of each farm or each MAF model. The biodynamic farms had lower total gross margins than their conventional neighbors and most of the MAF conventional farms (Table 4). Much of this difference was due to the smaller size and greater enterprise diversity of the biodynamic farms.

The biodynamic farms proved in most enterprises to have soils of higher biological and physical quality: significantly greater organic matter content and microbial activity, more earthworms, better soil structure, lower bulk density, easier penetrability, and thicker topsoil. The results of the soil chemical analyses were variable. On a per

**Table 4.** Annual total incomes, variable costs, gross margins, and total gross margins. \* MAF, representative conventional farm, on the basis of the MAF model; other abbreviations as in Table 1.

Farm enterprise	1988				1989				1990				1991				Average (1988 to 1991)						
	Bio		MAF		Bio		MAF		Bio		MAF		Bio		MAF		Bio		MAF				
	Con	Con	Con	Con	Con	Con	Con	Con	Con	Con	Con	Con	Con	Con	Con	Con	Con	Con	Con	Con			
<b>Market gardens</b>																							
Total income (NZ\$ ha <sup>-1</sup> )†	11768	22925	†	12594	24999	†	16178	13519	†	15836	13938	†	14094	18845	†	14094	18845	†	14094	18845	†	†	
Variable costs (NZ\$ ha <sup>-1</sup> )	4558	10875	†	4250	9754	†	5359	5894	†	5742	5832	†	4977	8088	†	4977	8088	†	4977	8088	†	†	
Gross margin (NZ\$ ha <sup>-1</sup> )	7210	12050	†	8344	15245	†	10819	7625	†	10094	8106	†	9117	10757	†	9117	10757	†	9117	10757	†	†	
Total gross margin (NZ\$)	36050	457900	†	41720	579310	†	54095	289750	†	50470	308028	†	45585	408766	†	45585	408766	†	45585	408766	†	†	
<b>Citrus orchards</b>																							
Total income (NZ\$ ha <sup>-1</sup> )	9231	8873	13750	12103	8635	10681	17725	14677	17035	14677	12459	13434	13434	13434	12459	13434	13434	13434	13434	13434	13481	13481	
Variable costs (NZ\$ ha <sup>-1</sup> )	4886	3669	9569	7317	3929	7288	5256	7556	9267	7556	9771	6254	6254	6254	9771	6254	6254	6254	6254	6254	6254	8974	8974
Gross margin (NZ\$ ha <sup>-1</sup> )	4345	5204	4181	4786	4706	3393	12469	7121	7768	7121	2688	7180	7180	7180	2688	7180	7180	7180	7180	7180	7180	4507	4507
Total gross margin (NZ\$)	14339	34867	25086	15794	31530	20358	41148	46608	46608	23499	16128	23694	23694	23694	16128	23694	23694	23694	23694	23694	27042	27042	
<b>Mixed farms</b>																							
Total income (NZ\$ ha <sup>-1</sup> )	706	1134	886	627	1360	994	742	1498	1278	738	1355	950	703	1337	1355	950	703	703	703	703	1027	1027	
Variable costs (NZ\$ ha <sup>-1</sup> )	303	456	305	298	526	405	357	590	551	288	575	484	311	537	575	484	311	311	311	311	436	436	
Gross margin (NZ\$ ha <sup>-1</sup> )	403	678	581	329	834	589	385	908	727	450	780	466	392	800	780	466	392	392	392	392	591	591	
Total gross margin (NZ\$)	76570	176280	90055	62510	216840	91295	73150	236080	112685	85500	202800	72230	74480	208000	202800	72230	74480	74480	74480	74480	91605	91605	
<b>Livestock farms</b>																							
Total income (NZ\$ ha <sup>-1</sup> )	472	301	274	442	345	295	420	432	404	516	495	339	463	393	495	339	463	463	463	463	328	328	
Variable costs (NZ\$ ha <sup>-1</sup> )	28	66	60	30	80	80	49	90	92	76	96	103	46	83	96	103	46	46	46	46	84	84	
Gross margin (NZ\$ ha <sup>-1</sup> )	444	235	214	412	265	215	371	342	312	440	399	236	417	310	399	236	417	417	417	417	244	244	
Total gross margin (NZ\$)	44400	102930	64200	41200	116070	64500	37100	149796	93600	44000	174762	70800	41700	135780	174762	70800	41700	41700	41700	41700	73200	73200	
<b>Dairy farm set 1</b>																							
Total income (NZ\$ ha <sup>-1</sup> )	1842	1089	1089	2020	1887	1468	2211	2211	1667	3060	1196	2283	2283	1196	1196	2283	2283	2283	2283	2283	1355	1355	
Variable costs (NZ\$ ha <sup>-1</sup> )	740	315	315	514	449	449	908	908	552	1169	389	833	833	389	389	833	833	833	833	833	426	426	
Gross margin (NZ\$ ha <sup>-1</sup> )	1102	774	774	1506	1019	1019	1303	1303	1115	1891	807	1450	1450	807	807	1450	1450	1450	1450	1450	929	929	
Total gross margin (NZ\$)	25346	61920	61920	34638	81520	81520	29969	29969	89200	43493	64560	33350	33350	64560	64560	33350	33350	33350	33350	33350	74320	74320	
<b>Dairy farm set 2</b>																							
Total income (NZ\$ ha <sup>-1</sup> )	1115	1365	1365	1381	1887	1781	2052	2289	2204	2237	2534	1916	1696	2237	2534	1916	1696	1696	1696	1696	1817	1817	
Variable costs (NZ\$ ha <sup>-1</sup> )	621	398	398	601	404	535	947	536	587	1503	568	531	918	503	568	531	918	918	918	918	513	513	
Gross margin (NZ\$ ha <sup>-1</sup> )	494	967	967	780	1483	1246	1105	1753	1617	734	1966	1385	778	1734	1966	1385	778	778	778	778	1304	1304	
Total gross margin (NZ\$)	111150	65756	65756	175500	222450	84728	248625	262950	109956	165150	294900	94180	175050	260100	294900	94180	175050	175050	175050	175050	88672	88672	

†The effective enterprise areas for calculation of total gross margins are as follows: Bio veg = 5 ha, Con veg = 38 ha; Bio cit = 3.3 ha, Con cit 2 = 6.7 ha, MAF cit = 6 ha; Bio mixed = 190 ha, Con mixed = 260 ha, MAF mixed = 155 ha; Bio livestock = 100 ha, Con livestock = 438 ha, MAF livestock = 300 ha; Bio dairy 1 = 23 ha, MAF dairy 1 = 80 ha; Bio dairy 2 = 225 ha, Con dairy 2 = 150 ha, MAF dairy 2 = 68 ha. †NZ = New Zealand; NZ \$1.00 = US \$0.60. ‡There are no MAF models for market gardens. §The farm was sold in 1990 and economic records for the new manager were not reliable for 1990 and 1991. ¶Farm began producing beef in 1989, a product not comparable to that of the dairy farm. ¶¶Farm was under a different manager than in 1989 to 1991.

hectare basis, the biodynamic farms were just as often financially viable as their neighboring conventional farms and representative conventional farms.

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- Ten pairs of paddocks were directly adjacent to each other; 5 to 6 soil samples were taken from each paddock. Two paddocks were in hill country and had to be sampled about 300 m apart to get the same slope and aspect; here 12 soil samples were taken from each paddock. Soil samples were collected in the spring of 1990 and the summer of 1990 to 1991 from the upper 10 cm.
- Soil samples were analyzed for the following properties: total carbon, with the use of a Leco (Saint Joseph, MI) high-frequency induction furnace; extractable potassium, calcium, and magnesium, with the use of a semimicro leaching procedure; pH in a water suspension; extractable phosphorus and cation exchange capacity as described in L. C. Blakemore, P. L. Searle, and B. K. Daly, *New Zealand Soil Bureau Scientific Report 80* (Department of Scientific and Industrial Research, Lower Hutt, New Zealand, 1987); soil respiration, by manometric measurements of the respiratory uptake of gaseous oxygen by soil [W. W. Umbreit, R. H. Burris, J. F. Stauffer, *Manometric and Biochemical Techniques* (Burgess, Minneapolis, 1972)] and modified by A. N. Macgregor and L. M. Naylor [*Plant Soil* **65**, 149 (1982)]; mineralizable soil nitrogen, by incubation [D. R. Keeney and J. M. Bremner, *Soil Sci. Soc. Am. Proc.* **31**, 34 (1967)]; total nitrogen and phosphorus, with the use of a micro-Kjeldahl digestion of soil followed by nitrogen analysis [Technicon, *Industrial Method No. 329-74 W/A* (Technicon, Tarrytown, NY, 1976)] and phosphorus analysis [J. R. Twine and C. H. Williams, *Commun. Soil Sci. Plant Anal.* **2**, 485 (1971)]; and sulfate, by the automated Johnson and Nishita technique [B. Hefner, *A Handbook of Methods of Inorganic Chemical Analysis for Forest Soils, Foliage, and Water* (CSIRO Division of Forest Research, Canberra, Australia, 1985)]. Soil profiles were analyzed in the field for the following properties: soil texture, structure, and consistence as described by standard New Zealand Soil Bureau procedures [N. H. Taylor and I. J. Pohlen, *Soil Bureau Bulletin 25* (Soil Bureau, Lower Hutt, New Zealand, 1962)]; bulk density with the use of thin-walled aluminum cores; and penetration resistance with the use of a Rimik (Toowoomba, Queensland, Australia) CP10 cone penetrometer.
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  - We thank the 16 New Zealand farm families for

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# Transient Transfection and Expression in the Obligate Intracellular Parasite *Toxoplasma gondii*

Dominique Soldati and John C. Boothroyd\*

*Toxoplasma gondii* is a protozoan pathogen that produces severe disease in humans and animals. This obligate intracellular parasite provides an excellent model for the study of how such pathogens are able to invade, survive, and replicate intracellularly. DNA encoding chloramphenicol acetyltransferase was introduced into *T. gondii* and transiently expressed with the use of three vectors based on different *Toxoplasma* genes. The ability to introduce genes and have them efficiently and faithfully expressed is an essential tool for understanding the structure-function relation of genes and their products.

*Toxoplasma gondii* is a ubiquitous parasite that can infect almost any warm-blooded vertebrate. In humans, it has long been recognized as a major cause of severe congenital disease. More recently, it has emerged as one of the most important opportunistic pathogens in patients with acquired immunodeficiency syndrome (AIDS) (1). In the laboratory, *T. gondii* is relatively easy to handle and maintain and consequently has become an important model for the study of how obligate intracellular parasites function. To date, however, such studies have been hampered by the absence of a method for introducing DNA into the parasites. In part, this lack has been due to the difficulty of transfecting one cell inside another: the many membranes that the transfecting DNA must cross represent a significant barrier, and the dependence on the host cell for survival can further preclude manipulations of the extracellular parasite. As a result, although transfection and stable transformation have been achieved for a range of trypanosomatids (2–8), such methodologies have not been reported for any of the obligate intracellular parasites, most notably members of the phylum Apicomplexa, which includes *Toxoplasma*, *Eimeria*, and *Plasmodium*, the causative agent of human malaria.

Electroporation has successfully been used to introduce DNA into many cell types. It is believed that pores are generated by reversible electrical breakdown of the

cell membrane. Recent studies have shown that immediately after electroporation, cells are sensitive to the osmolarity and ionic composition of the medium and that the use of a potassium phosphate-based electroporation buffer (cytomix) that resembles the cytosol's ionic composition considerably increases cell survival (9). We chose, therefore, to use such a buffer in our initial transfection studies rather than culture medium or phosphate-buffered saline, which contain sodium ions at concentrations that are detrimental to the cells. We found that electroporation of *T. gondii* in cytomix buffer gives an extremely good rate of cell survival: an average of ~80% of the parasites are capable of invading host cells after electroporation as compared with the same population of parasites not subjected to an electric pulse.

For use as a reporter construct, a plasmid (SAG1/2 CAT) was made containing the chloramphenicol acetyltransferase (CAT) gene (11) and the upstream and downstream sequences of the *T. gondii* major surface antigen gene, p30 or SAG1 (12) (Fig. 1). This was done by a two-step method. First, reverse polymerase chain reaction (PCR) (13) was performed with an SK+ Bluescript vector (Stratagene) containing the complete SAG1 gene with the use of primers that generate an Nsi I site at the second in-frame ATG and a Pac I site at the stop codon. Then, a CAT cassette with a Nsi I site embracing its ATG and a Pac I site encompassing its stop codon was generated by PCR and cloned into the corresponding Nsi I–Pac I sites of the SAG1 expression vector.

Electroporation of this construct into

Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305.

\*To whom correspondence should be addressed.