ELSEVIER

Biological Conservation 69 (1994) 191–197

0006-3207/94/$07.00

SHORT-TERM EFFECTS AND LONG-TERM AFTER-EFFECTS OF FERTILIZER APPLICATION ON THE FLOWERING POPULATION OF GREEN-WINGED ORCHID *Orchis morio*

Jonathan Silvertown,[a] Derek A. Wells,[b] Mike Gillman,[a] Mike E. Dodd,[a] Heather Robertson[c] & Ken H. Lakhani[d]

[a] *Department of Biology, Open University, Milton Keynes, UK, MK7 6AA*
[b] *former Nature Conservancy Council,* [c] *English Nature, Northminster House, Peterborough, UK, PE1 1UA*
[d] *Institute of Terrestrial Ecology, Monks Wood, Abbots Ripton, Huntingdon, UK, PE17 2LS*

(Received 19 March 1993; revised version received 2 September 1993; accepted 21 September 1993)

Abstract

The green-winged orchid Orchis morio *has declined dramatically in England due to loss of habitat through the agricultural improvement of old hay meadows. A range of replicated fertilizer treatments was applied over a six year period to plots in an old hay meadow containing* O. morio, *to determine short-term effects and long-term after-effects. The numbers of flowering spikes per plot were counted annually for 23 years, commencing two years before experimental treatment. Inorganic fertilizers returning equivalent amounts of N, P and Mg and 80% of K to that removed in the annual hay crop significantly decreased flowering spike numbers during the six years of treatment. The fertilizer treatments, overall, significantly decreased flowering spike numbers, although the rates of N applied were low by agricultural standards (e.g. 22–88 kg ha⁻¹ N). Two applications of P at a rate of 40 kg ha⁻¹ during the six year period greatly reduced flowering spike numbers, apparently permanently. Except in the case of the 40 kg ha⁻¹ P treatment, the decrease was closely correlated with the increase in hay yield following fertilization, suggesting that competition between* O. morio *and the rest of the vegetation was responsible. The effect of P on* O. morio *was out of all proportion to its effect on hay yield, suggesting that it may have been toxic to the orchid. In retrospect we conclude that fertilizer effects would have been easier to detect if all plants of* O. morio *had been counted and not just flowering plants whose numbers fluctuated greatly. Even so, we have detected detrimental effects of low levels of fertilizer, and hence any application of inorganic or organic based fertilizers to old meadows should be avoided where conservation of* O. morio *is an objective.*

Keywords: biological conservation, fertilizer, hay meadows, *Orchis morio*, population dynamics.

INTRODUCTION

The extent of semi-natural, species-rich grassland in lowland England and Wales has shrunk dramatically in the latter half of this century. In a review of grassland surveys covering England and Wales, Fuller (1987) concluded that only 4% of lowland grasslands remained unimproved, and that the conservation interest of even some of this small fraction had been damaged; the most significant threat to the remaining grassland was application of inorganic nitrogen fertilizers. These were applied to 79% of permanent grasslands in 1985 (Elsmere, 1986). Habitat loss due to ploughing and reseeding or conversion to arable are important and obvious threats to grasslands of conservation interest but agricultural improvement due to fertilizer application is more insidious because the damage this may do is not always apparent until it is too late. Old hay meadows, named *Centaurea nigra–Cynosurus cristatus* grassland (MG5) in the National Vegetation Classification (Rodwell, 1992), are particularly at risk from this form of agricultural improvement.

The green-winged orchid *Orchis morio* occurs in old meadows that have long remained free from improvement (Rodwell, 1992). This orchid was once 'one of the commonest of British orchids' (Summerhayes, 1968), occurring throughout England and Wales, but due to habitat loss and agricultural improvement it is no longer common. As long ago as 1965 a survey of 30 sites in Lincolnshire where *O. morio* had been recorded in the previous 20 years found that at least 12 sites had been destroyed by agricultural improvement (Ball, 1965).

In 1970, a fertilizer experiment was set up at Bratoft Meadow, Lincolnshire, to examine the effect of different forms and rates of fertilizer application on the conservation value of old meadows. Fertilizers were applied at a range of rates (see below for details) for a period of six years between 1972 and 1977, and the numbers of flowering spikes of *O. morio* were counted annually from 1970 to 1992. In this paper we use these data (1) to examine the short-term effects of fertilizer application on *O. morio* flowering numbers and (2) to look for any long-term after-effects. Even though most meadows have by now lost their conservation interest because of agricultural improvement, the first of these objectives is still relevant to the conservation of *O. morio* because many of the old meadows that remain are still in low-intensity agricultural production and are at risk from improvement. The second objective becomes increasingly relevant as the number of old meadows dwindle because of the possibility that *O. morio* populations may recover at sites where fertilizers were once applied only in small amounts.

METHODS

Site description

Bratoft Meadows (also known as Heath's Meadows, Bratoft) is a 2.2 ha area of MG5 grassland (Rodwell, 1992), situated in North Lincolnshire (National Grid Reference TF 484640). It has been owned and managed by the Lincolnshire Trust for Nature Conservation since 1968, in which year it was also designated a Site of Special Scientific Interest on the grounds that it was 'the best example of species-rich neutral grassland in North Lincolnshire'. The site is included in Ratcliffe (1977) and attention was first drawn to its importance by its large flowering population of *Orchis morio*. It is annually cut for hay in the third or fourth week of July and the aftermath is grazed periodically by cattle until the end of October. Apart from fertilizer treatments applied to experimental plots in a small part of the meadows (see below), neither inorganic fertilizers nor herbicides have ever been used. It is traditional practice occasionally to spread farmyard manure on this type of meadow, but none was applied in the 30 years prior to the Lincolnshire Trust's ownership. A single application of well-rotted manure was made to the meadows in 1981, but the experimental area did not receive this treatment (B. Wilkinson, Reserves Officer, Lincolnshire Trust for Nature Conservation, pers. comm.).

Experimental design

Sixty-four contiguous, permanent plots, each 1.83 × 9.14 m in size were marked out in June 1970. From 1970 to 1976 the plots were cut for hay in the usual way, but were afterwards fenced to exclude cattle and a second cut was taken at the end of October. In 1970 and 1971 the inorganic nutrient contents of samples from the hay were analysed at Merlewood Experimental Station by standard techniques. In 1972, seven fertilizer treatments and a control were allocated at random within eight complete blocks. The rates of fertilizer application were chosen to return the inorganic nutrients removed in the hay to different degrees (Table 1). The average amounts of nutrients removed in the hay in 1970 and 1971 were: 44 kg ha^{-1} N, 4 kg ha^{-1} P, 65 kg ha^{-1} K, 6 kg ha^{-1} Mg. Treatment 5 was applied once in 1972 and once in 1975. All other treatments were applied annually in the spring from 1972 to 1977. Hay yield was sampled and weighed from a strip 7.31 m × 0.91 m down the middle of each plot at both cuts each year from 1970 to 1974. Three-hundred grammes of fresh hay from each sample were oven dried to obtain weights of dry matter.

Treatments 1, 2 and 3 provide a series which, with the control (Treatment 8), represent four graduated levels of fertilizer application. Treatments 2 and 4 applied the same levels of all four nutrients, but with N

Table 1. Fertilizer treatments applied to experimental plots, expressed as a percentage of the same nutrients removed in the hay crop that was sampled in the two years before the start of fertilizer application

Treatments 1–4 and 6 were applied annually 1972–1977, Treatment 5 was applied only in 1972 and 1975. Nutrients were applied in inorganic form, unless otherwise indicated.

Treatment	N	P	Mg	K
1	50% (organic)	50% (organic)	50%	40%
2	100% (organic)	100% (organic)	100%	80%
3	200% (organic)	200% (organic)	200%	160%
4	100%	100%	100%	80%
5	0	1000%	0	0
6	0	0	0	123%
7	0	0	1000%	0
8	0	0	0	0

and P in organic form (hoof and horn, bonemeal and urea) in Treatment 2 and in inorganic form (Nitrochalk™, superphosphate, magnesium sulphate, potassium sulphate) in Treatment 4 (Table 1). The fence around the experimental area was removed at the end of 1976, since when the experiment was cut for hay in July and grazed in common with the rest of the meadow. Numbers of flowering spikes of *O. morio* in each experimental plot, and also in four 1.83 × 9.14 m permanent plots located at random along a diagonal across the meadow but outside the experimental area, were counted annually between 15 May and 1 June 1970–1992.

Statistical analysis

All statistical analyses were performed using GENSTAT 5. A preliminary ANOVA of fertilizer treatments, with the factor Year as a split plot, was used on the whole data set to compare the relative effects of time (Year) and fertilizer (Treatment) and Block on orchid counts. Counts were square root transformed and degrees of freedom were adjusted to allow for repeated measures. This involved the calculation of Box's ε, a statistic measuring the departure of the population covariance matrix from homogeneity (Maxwell & Delaney, 1990) and calculated by the REPMEAS procedure of GENSTAT. The degrees of freedom for the ANOVA of the year stratum were multiplied by the estimated ε to give a new and increased critical F-value. Perfect homogeneity would give a ε of unity. To remove the effects of year and spatial variation within Blocks (see below), the whole data set was also analysed by analysis of covariance (ANCOVA), with the mean number of spikes/plot in 1970/71 (i.e. pre-fertilization) as a covariate. Effects of treatments in individual years were analysed by ANCOVA of counts or transformed counts. Effects of fertilizer treatments on dry weight of hay in the first cut were analysed by separate ANOVAS for each year 1970–1974.

RESULTS

The preliminary repeated measures ANOVA on square-root transformed counts showed that Treatment

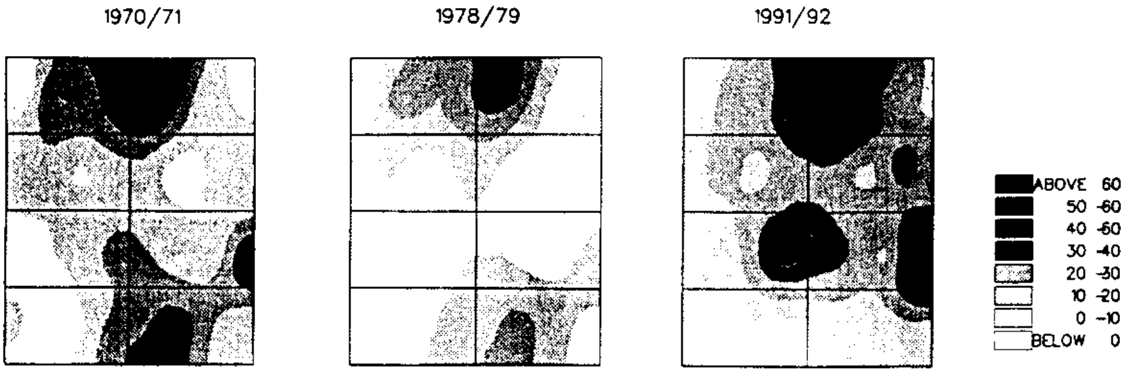

Fig. 1. Contour maps showing the mean numbers of spikes per plot in the experimental area in the two years before fertilization 1970/71, the two years following the cessation of fertilizer application in 1978/79, and in 1991/92. Block boundaries are shown.

had no significant effect ($F_{7,49}=1.98$), and that Year was highly significant ($F_{22,1232}=28.01$, $p<0.001$; $\varepsilon=0.1479$). This result is to be expected for three reasons: the populations fluctuated greatly over time, there was a trend of increasing spike numbers with time at the site, and fertilizers were applied over only a part of the period. The first two reasons are probably more important than the last because there was no Treatment × Year interaction ($F_{154,1232}=1.03$). To remove the effect of time on the results, each count was divided by the total number of spikes counted in the year and these proportions were then angular-transformed.

In the two years before treatment there was great spatial variation in orchid density across plots. Contour maps of the mean spike density/plot in 1970/71 and in the two years after treatments had ceased (1978/79) show that the similarity before and after treatment was great (Fig. 1), strongly suggesting that initial orchid numbers per plot might mask treatment effects. The original choice of a randomised block experimental design was intended to remove such spatial variation. However, Fig. 1 suggests that there was also spatial variation within Blocks. We therefore used the mean number of spikes/plot in 1970/71 as a covariate.

ANCOVA on angular-transformed proportional counts, using the mean number of spikes/plot in 1970/71 as a covariate, successfully removed the Year effect seen in the preliminary ANOVA, showed the covariate to be highly significant in the second stratum (within Blocks), and showed fertilizer treatments to be significant (Table 2). Mean numbers of orchid spikes per treatment over the period 1972–1992, adjusted for the covariate, are shown in Fig. 2. Treatments 3, 4 and 5 were significantly different from the control after spatial and temporal variation affecting spike numbers had been removed in the ANCOVA. Numbers of spikes in all the other treatments were lower than the control, but not significantly so (Fig. 2).

ANCOVAs, with count proportions angular transformed, were performed on the data of individual years (Table 3). In the two years pre-fertilization there was no significant difference between the plots destined to become controls and any set of plots destined to receive treatment. Counts averaged 13.2 spikes/plot in 1970 and 14.9 in 1971. The covariate was significant in all years 1972–1992, but its variance ratio (F, Table 3) decreased with time, indicating the diminishing effect of the initial distribution of orchid spikes.

Looking at the mean number of spikes per plot in different treatments through time, adjusted for the initial spike numbers (i.e. the covariate), the effect of fertilizer is clear comparing Treatments 1, 2 and 3 with

Table 2. Results of repeated measures ANCOVA
The significance of F values was determined for the appropriate d.f. after adjustment for repeated measures. Adjusted d.f. = d.f. × ε, where ε = 0·135 1. *, p < 0·05; **, p < 0·01; ***, p < 0·001; ns, not significant

Source of variation	d.f.	F	*p*
Stratum 1			
Block	6, 48	4·68	***
Covariate	1, 6	3·17	ns
Stratum 2			
Treatment	7, 48	3·84	**
Covariate	1, 48	66·11	***
Stratum 3			
Year	20, 1120	0	ns
Treatment × Year	140, 1120	0·56	ns

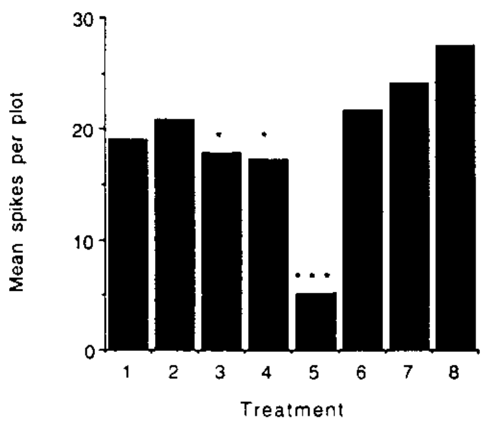

Fig. 2. Mean numbers of spikes per plot 1972–1992 in the seven treatments and control, adjusted for the initial number of spikes/plot before the experiment began. Asterisks indicate significant differences from the control (Treatment 8): *, p<0.05; ***, p<0.001.

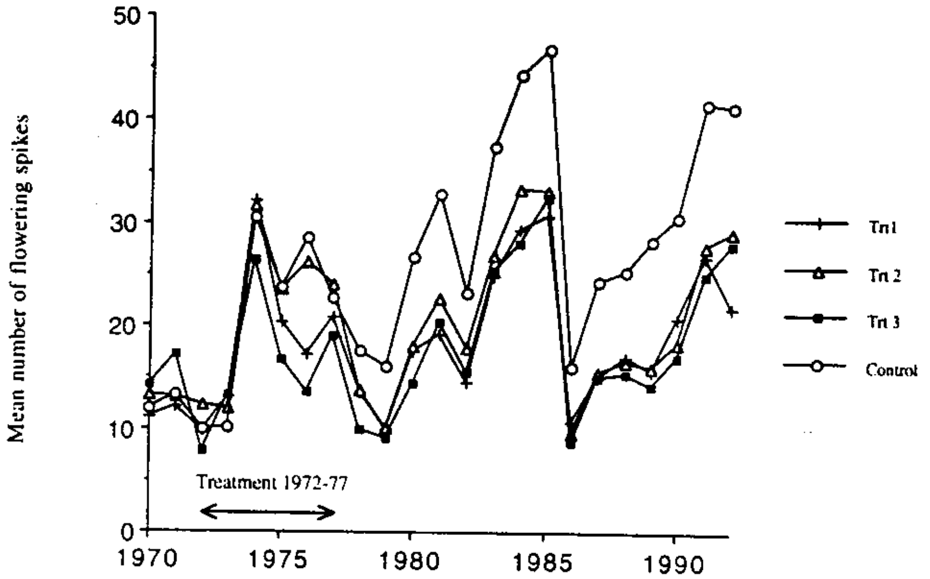

Fig. 3. Mean number of spikes per plot 1970–1992 in treatments receiving four levels of NPKMg.

the control. Treatments 1–3 (50–200% organic NP) behaved almost identically from 1978 to 1992, and their mean counts were always lower than the control (Fig. 3). Statistical analysis of these data, which of course allows for the variance within treatments that is not seen in Fig. 3, did not show the differences between Treatments 1–3 and the control to be significant (Table 3). The first significant effect of treatment appeared in the third year of fertilization (Table 3) when Treatment 5 (1000% P) had significantly fewer orchid spikes than the control (7.8 compared with 28.9) and a year later the count in Treatment 4 (100% inorganic NP) was also significantly depressed (10.9 compared with 27.5). From then on, Treatment 5 was permanently significantly lower than the control. The mean numbers of spikes in other treatments were consistently lower than controls over the whole post-fertilization period (Figs 2 and 3), but the differences were only occasionally significant for Treatment 2, once for Treatment 4, and never so for Treatments 1, 3, 6 and 7.

Comparing Treatment 2 receiving organic N and P with its inorganic counterpart Treatment 4 over the period of fertilizer application (Fig. 4), the mean number of spikes appears to fall more rapidly on the inorganic plot than on the organic one. However, Treatments 1 and 3 also received organic N and P, and behaved more like the inorganic Treatment 4 than the organic Treatment 2 (Fig. 3).

Analysis of variance of hay yield in 1970 and 1971 showed no significant differences between plots destined to receive different fertilizer treatments. Plots receiving Treatments 1–5 were all significantly different ($p<0.05$) and Treatments 6 and 7 were not significantly different from the control every year from 1972 to

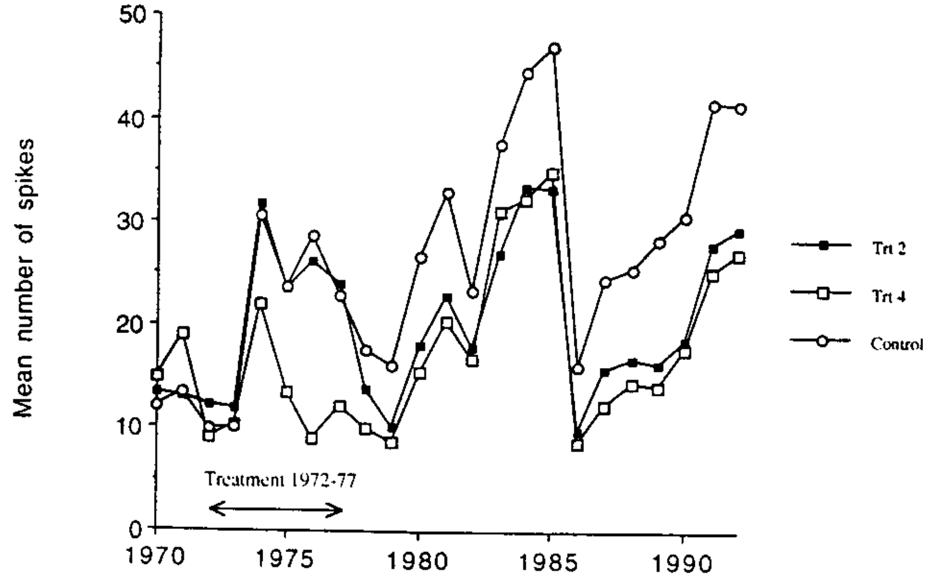

Fig. 4. Mean number of spikes per plot 1970–1992 in treatments receiving NP in organic (Treatment 2) and inorganic (Treatment 4) form, compared to the control. From 1972 onwards values were adjusted for the initial number of spikes/plot before the experiment began.

Table 3. Results of the ANOVA in 1970 and 1971 and the yearly ANCOVA 1972–1992, indicating which treatments were significantly different from the control in which years (*, $p < 0{\cdot}05$; **, $p < 0{\cdot}01$; ***, $p < 0{\cdot}001$)

Year	Treatment $F_{7,48}$	Covariate $F_{1,48}$	Treatments significantly different from control ($p < 0{\cdot}05$)	
1970	0·05	—	None	
1971	0·12	—	None	Pre-fertilization
1972	1·51	206·64***	None	
1973	2·55*	113·38***	None	
1974	7·18***	130·59***	5	Fertilizers applied
1975	8·29***	98·83***	4, 5	
1976	7·05***	64·16***	3, 4, 5	
1977	3·79**	65·74***	4, 5	
1978	5·88***	37·40***	5	Post-fertilization
1979	4·45***	36·05**	2, 5	
1980	4·24***	62·31***	4, 5	
1981	4·28***	45·44***	5	
1982	3·24**	35·24***	5	
1983	3·90**	38·42***	5	
1984	4·07***	28·37***	5	
1985	4·44***	33·14***	5	
1986	3·43**	12·42***	5	
1987	2·59*	18·58***	5	
1988	3·67**	16·92***	5	
1989	3·40**	14·07***	2, 5	
1990	2·95*	14·78***	2, 5	
1991	3·65**	12·42***	5	
1992	3·12**	9·97**	5	

1974. The relationship between mean flowering spike numbers per treatment 1972–1992, adjusted for the initial number of flowering spikes/plot before the experiment began, and the mean hay yield per treatment (kg ha^{-1} dry weight) for the last two years of hay sampling (1973–74) is shown in Fig. 5. The regression equation for the curve shown is: $y=30.76-0.003x$, $R^2=0.697$, $n=7$, $p<0.05$. Treatment 5 was a clear outlier from the regression and so was not included in it.

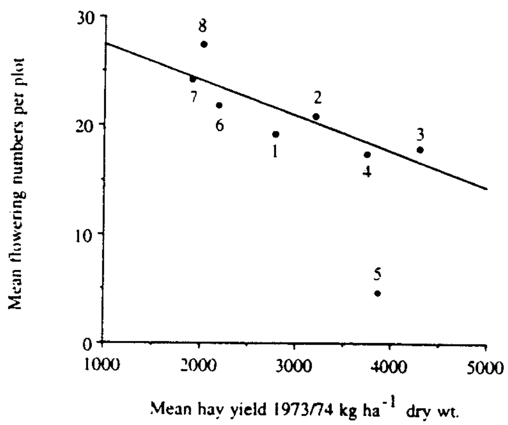

Fig. 5. Regression of mean flowering spike numbers per treatment 1972–1992, adjusted for the initial number of flowering spikes/plot before the experiment began, on the mean hay yield per treatment (kg ha^{-1} dry weight) 1973–74. The regression equation for the curve shown is: $y=30.76-0.003x$, $R^2=0.697$, $n=7$, $p<0.05$. Treatments are indicated by their numerals. Treatment 5 was not included in the regression.

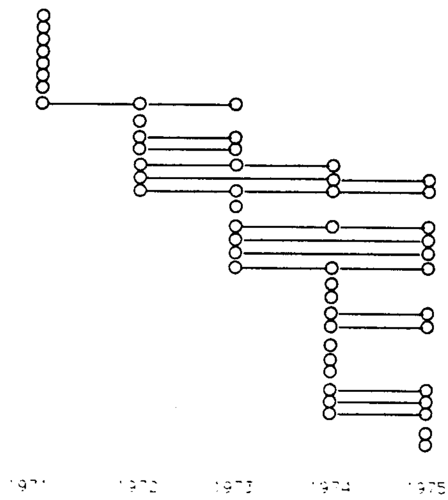

Fig. 6. Records of flowering (circles) for plants (lines) recorded at 31 locations mapped in three experimental plots between 1971 and 1975.

DISCUSSION

All fertilizer treatments had lower mean counts of orchid flower spikes than the control. However, variance between replicates was so high that the strong suggestion that *all* fertilizer treatments lowered spike numbers cannot be statistically confirmed on the basis of analysis of variance alone (Fig. 2). The results of the repeated measures ANCOVA showed that NPKMg fertilizers returning more nutrients than those removed in the hay crop (Treatment 3), or the same quantity, but in an inorganic form (Treatment 4) did significantly reduce the number of flowering spikes/plot of *Orchis morio* (Fig. 2). Although Treatment 2 (100% return of organic NP and inorganic Mg) did not prove significantly different from the control in the overall, repeated measures ANCOVA, numbers of orchid spikes were significantly depressed in this treatment in 1979 and also in 1989 and 1990, some 12 years after fertilizer application had ceased (Table 3). The significance of the delayed effects could be criticized on the grounds that we have made multiple comparisons (seven treatments × 12 years=84 possible comparisons), but we believe that at least the 1979 effect was a real one.

Treatments 2 and 4 were originally intended to allow a comparison of the effects of organic and inorganic sources of N and P applied at the same rate (100% return). One might expect the inorganic Treatment 4 to take effect earlier than the organic Treatment 2, and/or to have a larger effect because nutrients will have been more rapidly released from the inorganic than from the organic fertilizer. Both expectations are borne out. Treatment 4 had a significant negative effect from 1975 to 1977 (and again in 1980), while Treatment 2 had no significant effect until 1979 (Fig. 4 and Table 3). Treatment 4 was significant in the repeated measures AN-

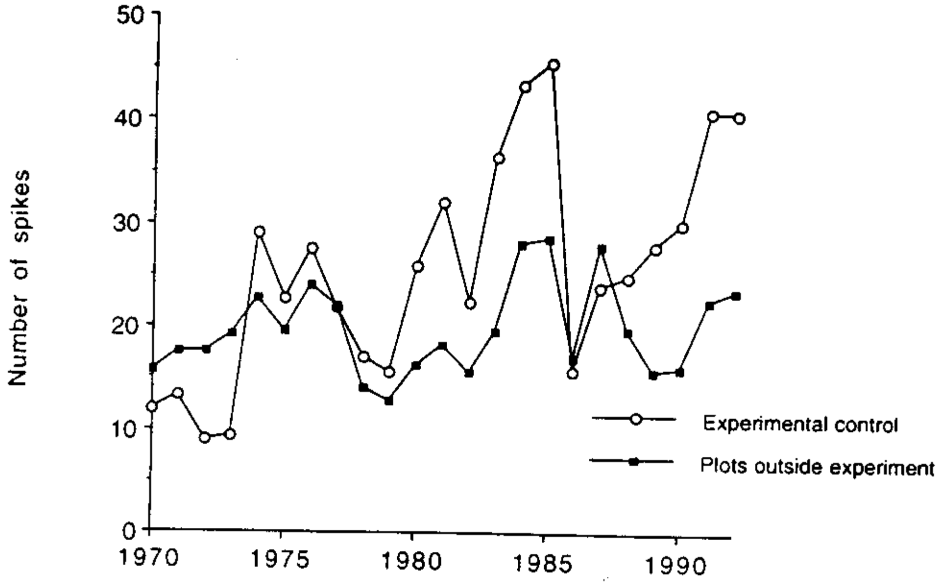

Fig. 7. Mean numbers of spikes per plot 1970–1992 in the experimental controls and in four plots of the same size outside the experiment.

COVA while Treatment 2 was not (Fig. 2). Over time, the means of organic Treatments 1 (50% organic NP) and 3 (200% organic NP) behaved more like the mean of the inorganic Treatment 4 than organic Treatment 2 (Figs. 3 and 4). However, there is no evidence that any effect of Treatment 1 was ever significant. That Treatment 3, like Treatment 4, had an earlier effect than Treatment 2 (1976, Table 3) and that its effect was significant in the overall repeated measures ANCOVA may be accounted for by the fact that it contained twice the amount of organic NP as Treatment 2.

The thousand per cent return of P in 1972 and 1975 had an almost immediate and apparently permanent effect in drastically reducing orchid numbers (Treatment 5, Fig. 2 and Table 3). This was to be expected in view of the amount applied and the immobility of P in soil, which prevents the leaching of this nutrient. Treatments 6 and 7 (123% K, 1000% Mg) seem to have depressed the mean number of spikes compared to the control (Fig. 2), but we did not find this to be statistically significant.

The relationship between mean hay yield and mean numbers of flowering spikes (Fig. 5) was a remarkably strong one and suggests that the effect of all fertilizer treatments, except 1000% return of P (Treatment 5), can be explained by their effects on the biomass of the community as a whole. Furthermore, this relationship confirms that the reduction in flowering numbers seen in all fertilizer treatments was probably a real one, even though not all treatments were significant in the ANCOVA (Fig. 2). *O. morio* is a plant of small stature, easily overtopped and shaded by grasses and other plants so it is easy to imagine why an increase in hay yield would reduce its flowering numbers. The drastic effect of Treatment 5 on flowering numbers cannot have been due solely to the effect of nutrients on hay biomass and interspecific competition because this treatment is so clearly an outlier from the regression of flowering numbers on hay biomass for all the other treatments. We suggest that 1000% return of P, which represents 40 kg ha^{-1}, was possibly toxic to the orchid or to its mycorrhizal symbiont.

Although we have detected a number of significant negative effects of quite low rates of fertilizer application (by agricultural standards) on *Orchis morio*, there is reason to believe that counts based on flowering spikes alone are not ideal for this purpose. In a study of the effects of grazing treatments on replicate populations of *Cirsium vulgare*, Gillman *et al.* (1993) found that treatment effects not detectable in simple counts of rosette numbers *were* detectable when population structure was analysed and rosettes were divided into size classes. There are also other reasons to suspect that the effects of all fertilizer treatments on the total population were probably larger than those we have shown on flowering spike numbers. The relationship of flowering plant numbers to total population numbers depends, in the first instance, upon the life cycle of the plant. *Orchis morio* is said to be quite short-lived, to be monocarpic, and to fluctuate strongly in numbers from year to year (Summerhayes, 1968). If this information is correct then one might expect flowering numbers and total numbers to be closely correlated, but this information is dubious. Censuses of mapped plants at all stages of the life cycle are really the only way to tell whether terrestrial orchids that disappear after flowering are really dead, or are still alive below ground and will later reappear (Hutchings, 1987). Flowering plants in a part of our experimental area were mapped. Figure 6 shows the continuity of flowering spikes at 31 locations mapped between 1971 and 1975. It is clear even from these few data that many plants were polycarpic, often flowering two years in a row. T. C. E. Wells (pers. comm.) has conducted a demographic study of *Orchis morio* and also found that plants at his site in Cambridgeshire are not monocarpic.

Wells and Cox (1991) mapped and censused *Ophrys apifera*, an orchid that, like *O. morio*, was reputed to be monocarpic, short-lived and fluctuating in numbers. They found that few plants in their study population were monocarpic, that individuals were quite long-lived and that total population numbers, which included plants that went 'missing' and then reappeared, fluctuated much less than an annual count of numbers flowering. Among other factors, one would expect year-to-year variation in the weather to affect flowering, particularly for an orchid near the edge of its geographical range in Europe. If *O. morio* is actually long-lived and polycarpic it is much easier to understand why its spatial distributions in 1970/71 and 1978/79 were so similar (Fig. 1), and why even 20 years later the spatial distribution of flowering spikes resembled the orginal one (Fig. 1), as testified by the fact that the covariate was still significant in our analysis (Table 3, 1992). From this circumstantial evidence, and by comparison with *O. apifera*, it seems very likely that the temporal variance in flowering numbers of *O. morio* in our experiment was probably much higher than the (unknown) variance in total population size must have been. This would have made treatment effects correspondingly more difficult to detect in our data than in total population counts. Any conclusion that a particular fertilizer treatment had no significant effect on flowering numbers should therefore not be interpreted to mean that the treatment had no effect on total population numbers. In the long term it is total population numbers that matter.

Spike numbers in the experimental controls and in four plots that were monitored on the reserve but outside the experimental area were correlated (r_{23}=0.627, p<0.01; Fig. 7) and show that the present experiment happened to coincide with a period of increase in the flowering population of *O. morio* at the study site. The same fertilizer treatments applied to a population in equilibrium or in decline might have had more severe effects. From the perspective of biological conservation one must take the view that all risks to threatened species should be minimized. The rates of fertilizer application used in our experiment were very low by modern agricultural standards, but even these levels increased hay yield by enough to reduce flowering numbers of *O. morio*. Therefore our experimental results suggest that, however limited, any application of inorganic or organic based fertilizers to old meadows should be avoided where conservation of *O. morio* is an objective.

ACKNOWLEDGEMENTS

We are grateful to the Lincolnshire Trust for Nature Conservation, and their Reserves Officer Barrie Wilkinson, for their co-operation and assistance throughout the study on their reserve and are indebted to the many people who helped with the work over the years, in particular Karen Buckley and John Blackwood. We thank James Bullock, Trevor Lambert and Kevin McConway for statistical advice and Terry Wells and Mike Hutchings for comments on the manuscript. The data analysis and production of this paper formed part of a contract carried out by The Open University for English Nature.

REFERENCES

Ball, M.E. (1965) Lincolnshire meadows and the green-winged orchid. *Newsl. Linc. Trust Nat. Conserv.*, November, 3–4.

Elsmere, J.I. (1986). Use of fertilizers in England and Wales, 1985. *Rothamsted Experimental Station Report for 1985*. 245–51. Harpenden, Lawes Agricultural Trust.

Fuller, R.M. (1987). The changing extent and conservation interest of lowland grasslands in England and Wales: a review of grassland surveys 1930–84. *Biol. Conserv.*, **40**, 281–300.

Gillman, M., Bullock, J.M., Silvertown, J. & Clear Hill, B. (1993). A density-dependent model of *Cirsium vulgare* population dynamics using field-estimated parameter values. *Oecologia, Berl.*, **96**, 282–9.

Hutchings, M.J. (1987). The population biology of the early spider orchid, *Ophrys sphegodes* Mill., II. Temporal patterns in behaviour. *J. Ecol.*, **75**, 729–42.

Maxwell, S.E. & Delaney, H.D. (1990). *Designing experiments and analysing data: a model comparison perspective*. Wadsworth, California.

Ratcliffe, D.A. (ed.) (1977). *A nature conservation review*. Cambridge University Press, Cambridge.

Rodwell, J.S. (ed.) (1992). *British plant communities, Vol. 3. Grasslands and montane communities*. Cambridge University Press, Cambridge.

Summerhayes, V.S. (1968). *Wild orchids of Britain*, 2nd edn. Collins, London.

Wells, T.C.E. & Cox, R. (1991). Demographic and biological studies on *Ophrys apifera*: some results from a 10-year study. In *Population ecology of terrestrial orchids*, ed. T.C.E. Wells & J. Willems. SPB Academic, The Hague, pp. 47–71.