precipitation, exchanged into 10 mM tris-HCl and 1 mM EDTA (pH 7.5) with a Bio-Rad p-10 column, and resolved by SDS–polyacrylamide gel electrophoresis (12% gel). The resolved proteins were electroblotted onto a polyvinylidene difluoride membrane; one lane of the sample (approximately 2% of the total protein) was immunoblotted with antibody to phosphotyrosine to localize p38, and the remainder of the blot was stained with Ponceau S. The stained p38 band was excised, and after treatment with trypsin (*29*), the resultant peptides were separated by high-performance liquid chromatography on a C18 column with a 0 to 100% acetonitrile gradient containing 0.01% trifluoroacetic acid (*29*). Fractions were collected, and a number of peptides were sequenced. All peptide sequences were found in the cDNA clone isolated. Degenerate oligonucleotides (16 mer) corresponding to either end of peptide 25, NFANVFIGANPLAVDLLEK (*15*), were used for the polymerase chain reaction (PCR) with cDNA from 70Z/3 cells as a template in order to obtain a DNA fragment containing the exact nucleotide sequences encoding the middle part of this peptide. Products of PCR resolved in an 8% acrylamide gel were eluted and subcloned into pBluescript vector. Sequencing verified the fidelity of the PCR product. A murine liver cDNA library (ClonTech, Palo Alto, CA) was screened with this PCR probe, and nine positive clones containing 0.8- to 3.5-kb inserts were obtained after screening 5×10^5 colonies. Restriction enzyme mapping and partial DNA sequencing indicated that all clones were identical; the longest cDNA clone was chosen for complete sequencing.

11. J. Han and R. J. Ulevitch, unpublished data.
12. The BLAST program was used to search the database through the National Center for Biotechnology Information. The PILEUP and PRETTYBOX programs were used for sequence alignments and comparisons. The FASTA program was used for calculating the identities of various protein kinases and of p38. The calculation of percent identity was done with 360 amino acids. All computer programs were from the Wisconsin Genetics Computer Group, Madison, WI.
13. S. K. Hanks, A. M. Quinn, T. Hunter, *Science* **241**, 42 (1988).
14. E. Nishida and Y. Gotoh, *Trends Biochem. Sci.* **18**, 128 (1993).
15. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
16. B. Derijard *et al.*, *Cell* **76**, 1025 (1994).
17. F. Zhang, A. Strand, D. Robbins, M. H. Cobb, E. J. Goldsmith, *Nature* **367**, 704 (1994).
18. D. Schild, A. J. Brake, M. C. Kiefer, D. Young, P. J. Barr, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 2916 (1990).
19. A. H. Ding, F. Porteu, E. Sanchez, C. F. Nathan, *Science* **248**, 370 (1990).
20. J.-D. Lee *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 9930 (1993).
21. C. L. Manthey, M. E. Brandes, P. Y. Perera, S. N. Vogel, *J. Immunol.* **149**, 2459 (1992).
22. S. B. Corradin, J. Mauel, R. J. Ulevitch, P. S. Tobias, *J. Leukocyte Biol.* **52**, 363 (1992).
23. R. J. Davis, *J. Biol. Chem.* **268**, 14553 (1993).
24. C. A. Lange-Carter, C. M. Pleiman, A. M. Gardner, K. J. Blumer, G. L. Johnson, *Science* **260**, 315 (1993).
25. F. A. Gonzalez, D. L. Raden, M. R. Rigby, R. J. Davis, *FEBS Lett.* **304**, 170 (1992).
26. T. G. Boulton *et al.*, *Cell* **65**, 663 (1991).
27. B. C. Tilly, N. van den Berghe, L. G. J. Tertoolen, M. J. Edixhoven, H. R. de Jonge, *J. Biol. Chem.* **268**, 19919 (1993).
28. M. Gustin, personal communication.
29. J. Fernandez, M. DeMott, D. Atherton, S. M. Mische, *Anal. Biochem.* **201**, 255 (1992).

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TECHNICAL COMMENTS

Biological Control and Refute Theory

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In classical biological control, biologists introduce an imported parasitoid in order to reduce the density of an accidentally imported insect host. If the pest density declines and the introduced natural enemy achieves moderate-to-high frequencies of attack, projects are considered successful. An association between successful biological control and high parasitization is to be expected. Hawkins *et al.* (*1*) found that maximum parasitization rates occurred when hosts had little protection from parasitoids (that is, small refuges), and they conclude (*1*, p. 1430) that susceptibility to parasitism (that is, high rates of parasitism) is a "significant estimate of the probability that the parasitoid introduction will reduce host densities." We find this conclusion suspect for five reasons.

1) The literature used by Hawkins *et al.* is likely to be biased in favor of the refuge hypothesis because cases of pest populations declining with low rates of parasitization are unlikely to be recorded as examples of successful biological control (*2*).

2) Refuge size is estimated by Hawkins *et al.* as one minus the proportion of the pest population parasitized. By definition, high parasitism characterizes successful biological control, therefore the argument that small refuges favor the success of biological control is a tautology.

3) Most simple measurements of "percent parasitism" contain errors or distortions arising from the influences of host and parasitoid phenologies (*3*). Single values cannot characterize host-parasitoid interactions, and maximum values are unlikely to be typical over longer times or in different areas (*4*).

4) Contrary to the prediction made by Hawkins *et al.*, successful biological control can result from the use of agents that are characterized by low rates of parasitization in their native habitat (*5*). Natural enemies that are rare in their native habitat may have superior potential as control agents when released in exotic habitats (*6*).

5) Hawkins *et al.* attribute seven cases of high parasitization (above 60%) in unsuccessful biological control projects to climatic mismatch between parasitoids and hosts. We question how such high rates of parasitization could be achieved if climatic factors "reduce parasitoid reproduction, survivorship, or host synchrony ..." (*1*, p. 1431).

Variation in the susceptibility of insects to predators, parasitoids, and disease is important. Mechanisms for encapsulating parasitoids, hiding from predators, and resisting disease influence the impacts of natural enemies in native and exotic habitats, but measuring refuge size from the observed maximum parasitization of successful biological control programs does not yield new understanding or predictability to the practice of biological control.

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REFERENCES

1. B. A. Hawkins, M. B. Thomas, M. E. Hochberg, *Science* **262**, 1429 (1993).
2. B. P. Beirne, *Can. Entomol.* **107**, 225 (1975).
3. R. G. van Driesche, *Environ. Entomol.* **12**, 1611 (1983).
4. A. McPhee, A. Newton, K. B. McRae, *Can. Entomol.* **120**, 73 (1988); I. A. Pearsall and S. J. Walde, *Ecol. Entomol.* **19**, 190 (1994).
5. J. Roland, in *Population Dynamics of Forest Insects*, A. D. Watt, S. R. Leather, M. D. Hunter, N. A. C. Kidd, Eds. (Intercept, Andover, United Kingdom, 1990), pp. 289–302.
6. J. Myers, C. H. Higgins, E. Kovacs, *Environ. Entomol.* **18**, 541 (1989)

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Hawkins *et al.* (*1*) demonstrate a significant and robust relationship between the outcome (success or failure) of a potential biocontrol program and the maximum percentage parasitism achieved by the parasitoid agent following its initial release. They propose that this relationship illustrates that the size of a "ref-

uge" against parasitism is inversely proportional to the probability of effective control in the long term. While we support the idea that refuge size may well be important in determining the depression of the host population achieved by a parasitoid, we contend that the data they present do not measure refuge size in the sense most frequently met in ecological studies.

A key assumption in the report by Hawkins *et al.* is that a constant fraction of the pest population is contained within a refuge regardless of host or parasitoid density; thus, inundation of the host population with the parasitoid will leave only those contained within the refuge unparasitized (*1, 2*). Host density is therefore unimportant and parasitoid density must be maximal. However, if this assumption is contravened, refuge size will depend on host density, parasitoid density, or both (*3, 4*). Refuges commonly encountered in the field result from diverse sources and many of these would result in a variable rather than a fixed proportion of hosts escaping attack. If attack rates follow a Poisson or negative binomial distribution (*3, 5, 6*), for example, then the probability of escaping attack decreases as the parasitoid:host ratio increases. Consequently, at extremely high parasitoid densities (such as those found at the epicentre of release or following exponential population growth of an exotic parasitoid), such a probability refuge is at a minimum. However, once the parasitoid is established and the host population is depressed, this ratio (and therefore the size of the probability refuge) will change. It is at this point that refuge size will determine the ultimate success of a biocontrol release.

Although Hawkins *et al.* obtain a strong relationship between their two variables, this indicates only that maximum percentage parasitism is a good indicator of the potential success of a biological control program. The data they present came from pest populations before parasitoid-induced depression, and includes data from release epicentres. However, percentage parasitism only signifies that the parasitoid chosen has become established. Establishment is the result of a number of parasitoid-related factors including (i) compatability with the pest, (ii) climatic matching to the release site, and (iii) release of sufficient numbers to prevent stochastic events causing extinction before establishment. Percentage parasitism will also indicate if there are any absolute refuges. Other common forms of host refuge will not be detected by snapshot samples of parasitism at periods of high parasitoid density.

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REFERENCES

1. B. A. Hawkins, M. B. Thomas, M. E. Hochberg, *Science* **262**, 1429 (1993).
2. M. E. Hochberg and B. A. Hawkins, *ibid.* **255**, 973 (1992).
3. M. P. Hassell, *The Dynamics of Arthropod Predator-Prey Systems* (Princeton Univ. Press, Princeton, NJ, 1978).
4. J. R. Beddington, C. A. Free, J. H. Lawton, *Nature* **273**, 513 (1978).
5. R. M. May, *J. Anim. Ecol.* **47**, 833 (1978).
6. _____ and M. P. Hassell, *Philos. Trans. R. Soc. London Ser. B* **318**, 129 (1988).

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Response: What kind of data are necessary to predict the outcome of parasitoid introductions for biological control? Myers *et al.* raise several issues as to why maximum parasitism rates should (or should not) predict the ultimate success or failure of a project. Their first two points propose that the relationship is tautological. However, the data included in our analysis judged the success of projects on the basis of economic criteria (*1*). An introduction that does not reduce the status of a pest will not be judged a success, irrespective of maximum parasitism rates. The reluctance of biocontrol workers to accept maximum estimates of parasitism as characterizing parasitoid-host interactions is argued by Myers *et al.* themselves in their third point.

In evaluating success in biological control and the underlying theory, it is important to distinguish between mean and maximum rates of parasitism. The former will include the effects of density-dependent factors and in most cases will be influenced by spatial and temporal variability in the susceptibility of the host to parasitoid attack. The latter measure minimizes the influence of such factors by estimating the susceptibility of the host when parasitoid densities are not limiting. There is a well-developed theory founded on variability in rates of parasitism (*2*), but the data suggest that quantifying this variability is not necessary for evaluating the impact of parasitoids on host populations. This is the unexpected aspect of our results.

Refuge theory does not predict that low parasitism *never* leads to reductions in host densities. The actual refuge level at which parasitoids can reduce host densities depends on host reproductive rate as well as the parasitoid's host utilization rate. On the other hand, the very low rates of host reproduction necessary for control to be effected by low rates of maximum parasitism are probably an uncommon feature among insects that achieve pest status, so the probability of success at low refuge levels should be small. This is what the data indicate.

The question of whether parasitoids that are rare in their native locations can reduce host densities after introduction was not addressed in our report, but it is an important issue. If by "rare," we mean that the parasitoid occurs at low densities, then it is indeed possible that it is an effective agent, because by keeping its host at low densities, it also occurs at low densities. If "rare" means that parasitism rates are very low, this may not have anything to do with the host if competition among parasitoids in a multispecies complex or hyperparasitism keeps that particular species rare. Either way, rarity does not tell us anything about host refuges. However, a related issue is whether low rates of maximum parasitism in the host's native range can be used to predict whether any parasitoid can affect control in exotic locations. If the current data are representative, they suggest that hosts that never suffer parasitism of more than 30% in their native ranges will be not amenable to biological control by using parasitoids. Thus, an additional test of the hypothesis would examine maximum (not mean) parasitism rates by all parasitoid species in native locations and success rates in exotic locations.

Refuge theory assumes that parasitoids are climatically adapted to the region of introduction. If not, our theory of host-parasitoid interactions based on dynamic constructs is not applicable. Examples of high maximum parasitism rates that do not lead to reductions in pest densities because of climatic mismatch have been given (*1*).

Williams and Hails raise the issue (from a theoretical perspective) of variability in parasitism rates resulting from density-dependent forces. As they summarize, much of the theory of parasitoid-host dynamics, including our own, suggests that this variability is important. Even so, our principal result indicates that quantifying such probabilistic refuges may not be critical in practice. Maximum percentage parasitism reflects more than just parasitoid establishment (which, although essential, provides no guarantee of successful control). It is an estimate of the minimum fraction of hosts escaping parasitism, or of the proportional refuge.

Finally, refuge theory offers a parsimonious explanation for a wide range of patterns found in parasitoid-host interactions, including success in biological control (*3*). Further empirical research will tell us the extent to which proportional refuges can serve as a predictor of these patterns.

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REFERENCES

1. B. A. Hawkins, M. B. Thomas, M. E. Hochberg, *Science* **262**, 1429 (1993).
2. M. P. Hassell, *The Dynamics of Arthropod Predator-Prey Systems* (Princeton Univ. Press, Princeton, NJ, 1978); R. M. May, *J. Anim. Ecol.* **47**, 833 (1978); S. W. Pacala, M. P. Hassell, R. M. May, *Nature* **344**, 150 (1990).
3. B. A. Hawkins, *Pattern and Process in Host-Parasitoid Interactions* (Cambridge Univ. Press, Cambridge, UK, 1994).

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Calcium and Inositol 1,4,5-Trisphosphate–Induced Ca^{2+} Release

One of the pathways that regulates cytosolic free Ca^{2+} is that of inositol 1,4,5-trisphosphate ($InsP_3$) inducing intracellular stores to release Ca^{2+} (*1*). It has been suggested that the dependence of this release on the concentration of free Ca^{2+} in the cytoplasm comprises Ca^{2+}-induced activation of the $InsP_3$-gated channel in the presence of submicromolar concentrations of cytosolic Ca^{2+} (*2*, *3*). The immediate positive feedback control of $InsP_3$-induced Ca^{2+} release resulting from such activation would be important in the regulation of store discharge, and this control has been incorporated into different models describing the spatiotemporal aspects of Ca^{2+} signaling, for example, hormone-induced oscillation in cellular Ca^{2+} and propagation of Ca^{2+} waves (*1–3*).

Nevertheless, as the buffering of free Ca^{2+} at low concentrations generally involves the use of Ca^{2+} chelators, the validity of such experiments may be doubted if the chelators have any activity other than chelating. Recently, Richardson and Taylor showed that several chelators interfered with the $InsP_3$ receptor (*4*). In their experiments, the Ca^{2+}-free forms of the Ca^{2+} chelator BAPTA and the related fura-2 dye proved to be competitive antagonists of $InsP_3$ binding to its receptor. In contrast, EGTA [another Ca^{2+} chelator that has been widely used in most of the experiments supporting Ca^{2+} activation of the $InsP_3$-gated channel (*2*)] was found by Taylor and Richardson to have virtually no effect on $InsP_3$ binding to its receptor, as measured at low temperature under alkaline conditions (*4*).

We further investigated the effect of EGTA by directly measuring $InsP_3$-induced $^{45}Ca^{2+}$ efflux from microsomal stores derived from cerebellum, a tissue rich in $InsP_3$ receptors. We found that 1 mM EGTA greatly reduced the amount of $^{45}Ca^{2+}$ released during 2 s (Fig. 1). The dose dependence of $InsP_3$-induced $^{45}Ca^{2+}$ release was studied in two different media buffered at pCa 6.5 (20°C, pH 7.1), which contained either 30 μM Ca^{2+}-free EGTA and 30 μM CaEGTA or 1 mM Ca^{2+}-free EGTA and 1 mM CaEGTA. In the presence of the higher concentration of EGTA, the dose-response curve for $InsP_3$ was shifted upwards by one order of magnitude, which implies inhibition by EGTA. Artifactual effects of EGTA or CaEGTA on different systems are well documented (*5*).

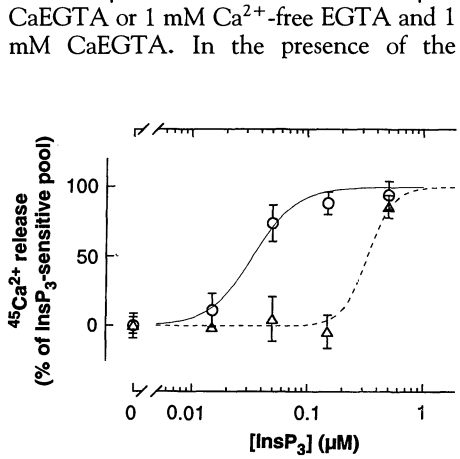

Fig. 1. Inhibition by EGTA per se of $InsP_3$-induced $^{45}Ca^{2+}$ release within 2 s from cerebellar microsomes. Microsomes, prepared from sheep brain (*7*), were actively loaded with $^{45}Ca^{2+}$, diluted in an $InsP_3$-free medium at pCa 9, and layered onto a nitrocellulose 0.45-μm filter. Most of the external $^{45}Ca^{2+}$ was washed out by a short rinse with the same $InsP_3$-free medium. The adsorbed microsomes were perfused with 2 ml of release medium containing different concentrations of $InsP_3$. Perfusion under controlled vacuum took about 2 s. The filter was counted with no additional rinsing, so that the perfusion-dependent drop in counted $^{45}Ca^{2+}$ reflected only the drop in trapped $^{45}Ca^{2+}$ during the 2-s perfusion. This drop is expressed as the fraction of the $InsP_3$-sensitive $^{45}Ca^{2+}$ pool released. The release medium contained 100 mM KCl, 20 mM NaCl, 5 mM $MgCl_2$, 25 mM Hepes-KOH (pH 7.1, 20°C), and either 30 μM EGTA plus 30 μM CaEGTA (○), or 1 mM EGTA plus 1 mM CaEGTA (△). In both cases, pCa was close to 6.5, as checked by fluorescence measurements after addition of fluo-3. The mean value, ± standard deviation, is indicated.

Preliminary experiments suggested that when the inhibitory effect of EGTA was taken into account, free Ca^{2+} was a poor activator of $InsP_3$-induced Ca^{2+} release (not shown), which is consistent with previous results by Meyer and co-workers (*6*). They found no influence of free Ca^{2+} in the range of 150 to 800 nM on the kinetics of Ca^{2+} release from permeabilized basophilic leukemia cells into Ca^{2+}-depleted media in which the only buffer for Ca^{2+} was fluo-3, present at a small concentration, around 1 μM. Do these results negate the activating effects of Ca^{2+} on $InsP_3$-induced Ca^{2+} release described previously? At least, they call for reexamination of the possible artifactual effects of Ca^{2+} chelators or Ca^{2+} probes in those experiments, as such effects likely account for part of the previously observed activation by Ca^{2+}. The Ca^{2+} sensitivity of the $InsP_3$ receptor might well have been overestimated.

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REFERENCES

1. C. W. Taylor and I. C. B. Marshall, *Trends Biochem. Sci.* **17**, 403 (1992); K. Mikoshiba, *Pharmacol. Sci.* **14**, 86 (1993); M. J. Berridge, *Nature* **361**, 315 (1993).
2. E. A. Finch, T. J. Turner, S. M. Goldin, *Science* **252**, 443 (1991); I. Bezprozvanny, J. Watras, B. E. Ehrlich, *Nature* **351**, 751 (1991); L. Missiaen, H. De Smedt, G. Droogmans, R. Casteels, *J. Biol. Chem.* **267**, 22961 (1992).
3. M. Iino and M. Endo, *Nature* **360**, 76 (1992); Y. Yao and I. Parker, *J. Physiol.* **458**, 319 (1992).
4. A. Richardson and C. W. Taylor, *J. Biol. Chem.* **268**, 11528 (1993).
5. G. B. Segel, W. Simon, A. W. Lichtman, M. A. Lichtman, *ibid.* **256**, 6629 (1981); T. L. Trosper and K. D. Philipson, *Cell Calcium* **5**, 211 (1984); M. A. Birch-Machin and A. P. Dawson, *Biochim. Biophys. Acta* **855**, 277 (1986).
6. T. Meyer, T. Wensel, L. Stryer, *Biochemistry* **29**, 32 (1990).
7. M. Hilly, F. Piétri-Rouxel, J. F. Coquil, M. Guy, J. P. Mauger, *J. Biol. Chem.* **268**, 16488 (1993).

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Response: After examination of Combettes and Champeil's legitimate concerns, we conclude that artifactual effects of Ca che-