Shortcomings in the Classic Tests of Candidate Insects for the Biocontrol of Weeds

by
P. H. Dunn[1]

ABSTRACT

Some of the testing of candidate insects for the biological control of weeds gives data of questionable value. Several views and criteria of testing are presented, which, if adopted, may shorten the pre-introduction and quarantine testing time without appreciably increasing the possibility of introducing a pest instead of a beneficial insect. Open cage testing, tests in gardens of beneficial plants, and the value of the literature in selecting candidate insects are considered.

INTRODUCTION

We are all familiar with the reality that there are enormous problems with weeds on every continent and that they are getting worse and that costs of control with chemical herbicides would have to be calculated in mega-dollars. These are some of the reasons why we are at this symposium to exchange findings and ideas. I would like to do the latter.

The thesis of this talk is that the classic safety tests in selecting candidate insects for the biological control of weeds could be improved from the point of view of efficiency. They are adequate from the safety aspect as the history of our work bears out. The principal limitation is that they produce much meaningless data that we try to integrate into our conclusions, sometimes creating confusion instead of clarity. Our literature is full of statements by workers saying that starvation and oviposition tests in cages expand the apparent host range or number of plants on which an insect can be made to feed, oviposit and complete larval development (Force 1966; Frick and Andres 1967; Andres and Angalet 1963).

By continuing to plod in the same footsteps, we have created a bottleneck. The weed problems are there, the phytophagous agents that could provide solutions to many of the problems exist, and we have the "go ahead" from our respective governments and agencies because our field and our product are recognized as valuable, workable and even desirable. The problem is that we are not up to the task of screening sufficient insect species to meet our needs because we often test the wrong things producing results that, when interpreted incorrectly, obscure valid conclusions regarding the safety of candidate species being tested. Such conclusions could and do result in condemnation and abandonment of some safe and useful species.

The responsibility for this current inadequacy is about equally divided between the investigators and the review boards who must make the ultimate decision on whether or not to permit an insect to be released. The investigator, even with an insect he feels is safe, must include all the data when the petition for introduction is sent for review. He may even include meaningless data, e.g., that a caged insect may feed on a plant that it would not be associated with in uncaged conditions. Such data become harmful because they can be interpreted negatively.

For example, *Psylliodes chalcomera* Illiger, a flea beetle candidate for the biological control of musk thistle (*Carduus nutans* L.) is not a pest of artichoke in Europe, but when one cages it with artichoke it can be made to feed and ovipost on it and a few progeny will mature. Since some individual insects can mature on artichoke it seemed useless to prepare a petition requesting permisison to release the species because it was felt that permission would be refused.

Studies in closed cages often produce the same information: if the insect is caged it can reproduce somewhat, but if freed on or near artichoke the insects ignore it. All of the evidence except the trials in cages (both starvation and field) show the insect is not a pest of artichoke. The dilemna is how to test the insect. This paper is written largely with this insect, the experience and possible solutions, in mind and is, in a sense, a question before the group.

The reviewers, not having participated in the first round of testing, naturally feel a little vulnerable in their position of having to rely on data

[1]Biological Control of Weeds Laboratory, USDA, ARS, 1050 San Pablo Avenue, Albany, CA. 94706.

provided them and are inclined to be super-prudent. They may even extrapolate the meaningless data into negative findings and not allow introduction, or request additional work which, if done in the same old way, may generate more useless data that will result in condemnation of the insect.

The necessity for urgent solutions to current weed problems has put investigators under pressure to use their imaginations and abilities as scientists to look for and discover more expeditious methodology to prove the safety or non-safety of candidate insects, and the review boards are under the same pressure to be receptive to new methodologies which prove candidate insects safe and effective. Since we are all on the same side, after the same goal, and frightened of the consequences of a mistake, it would seem proper for the review boards to participate in the research planning to have a better feel of the problems of the investigators, a hand in the solution of these problems, and thus feel more at ease at decision time.

The core of this discussion is to de-emphasize the importance of a certain part of the starvation and oviposition trials conducted in small cages to prove the specificity of an insect and to place a stronger emphasis on the literature and on field behavior, host selection and special adaptations as suggested by Zwölfer and Harris (1971).

LITERATURE

In those countries with a long and literary agricultural and entomological history, much credence can be given to the literature in selecting and clearing candidate insects, and the literature may be regarded as a two-edged weapon in our struggle. Pest insects are condemned before trial on the basis of the literature. Perhaps we should accept as safe an insect that is not incriminated by the literature just as quickly as we condemn it.

That idea is of course exaggerated but not completely without merit when one sees that the agricultural and entomological literature of the developed countries may be considered an enormous wide-ranging field trial conducted over many years and that the little trials we make in the laboratory are insignificant beside the contents of the literature. Thus, the literature is probably the best information we have on the safety or danger of species proposed for introduction from one country to another and is an invaluable guide when selecting candidate insects.

TESTS

Starvation: single plant

When our discipline was in its infancy, a starvation trial seemed an obvious and foolproof method of determining if an insect were safe to introduce or not. The idea commenced to fall apart in the late 1920's in the *Cactoblastis* program in Australia when it was found that caged *Cactoblastis* would also feed and sustain itself for some days on tomato fruits (Williams 1954).

Now that our discipline is maturing, we are finding again and again that by caging insects we can sometimes disrupt their natural behavior, causing them to feed and survive on such a ludicrous variety of plants that there is no way the findings can be transformed into meaningless and useful information. For example, 100 first-instar larvae of the spurge hawkmoth (*Hyles euphorbiae* [L.]), known to be specific to *Euphorbia* spp., produced 3 healthy adults when caged with Dalatian toadflax (*Linaria dalmatica* [L.] Mill.) as a sole food source. The startling thing is that *Euphorbia* and *Linaria* are not just in different families but completely different orders, i.e., Geraniales and Tubiflorae (Lawrence 1951).

To put and keep small cage starvation and oviposition tests in their proper perspective, I propose three guidelines. They are: (1) Always use a caged control of the host plant of the insect being tested to be sure the rearing conditions are not the cause of any non-feeding or mortality that may appear in the tests. This is important, because the dead insects are the important result of this kind of experiment.

(2) As a guide for the selection of plants, use the "centrifugal system" proposed by Wapshere (1974) plus any plants that may have a particular significance for the tests at hand.

(3) The test should be stopped when it is evident that the insects on some or all of the plants have died because they cannot or will not feed or did feed and were poisoned by the test plants, even though some insects may be alive and feeding well on some other test plants and in the control. At this point any mated in the tests should be examined for oogenesis; thus the useful information will have been obtained from the trial, and the plants with which the insect has a serious and evident incompatibility may be eliminated from further testing. By finding these incompatible plants, one has used the starvation trial to its fullest, and allowing it to go on because of continued feeding by some of the insects on the test plants generates useless data because there is no way to separate

the cage-induced feeding from what might be called uncaged feeding behavior. Those plants on which the insects survived are the basis for, and are included in, the next step in testing.

When the starvation test is used in this manner, the mechanisms that will prohibit insect attack on a useful plant can be separated into 2 categories; (1) nutritional incompatibility (e.g., toxic to the insect) and (2) unacceptability to the host finding mechanism of the insect.

Starvation: multiple plant

Frick (1974) described a possible second-step cage trial, a multiple choice test in which two or more plants in the same cage can be used to test preference of the insect for feeding and/or oviposition. A host plant may or may not be in the cage. While the idea is interesting, it does not seem that the information derived from such a trial would contribute important knowledge about the specificity of the insect, especially in tests when no host is included, because it cannot leave the plant so it has only the choice of feeding or not feeding. If all the insects die, the trial has yielded good information. However, the trial has some use if only a small number of insects are available for testing, because preliminary indications of incompatibility can be obtained by removing any plants attacked by the insects until all plants are removed or the insects starve on the remaining plants.

Field tests

The second step proposed here has two parts, both field tests, which must be conducted where the candidate is indigenous or has been liberated.

In 1968, Harley, in studying two beetles for introduction into Australia against *Lantana*, described 2 field trials that could have been the forerunner of those that will be considered here. For one, he proposed that large field cages be placed over infested target plants and then potted test plants be introduced into the cage so the insects could have multiple choice of plants under field-like conditions. The other test he described was done without cages by inter-planting test plants among weed host plants. These tests were done in Hawaii where the candidate insects had been established and there were high populations on the target weed.

Cage tests

The first test proposed here is different from the Harley cage test in 2 very important ways. 1. The target weed or control is not included in the cages with the test plants but, like them, is set up in its own separate cage. 2. The cage is 20% open on each of the four sides and the top. The rationale behind this design is simply that in closed cage testing the test insect is being restrained so that even if a plant is not a recognizable host, the pressure to feed overrides the more subtle pressures that normally prevent the insect from stopping on the plant. When this happens, the host selection system of the insect goes awry, it will commence maintenance feeding, and if the plant is not toxic, may continue to feed. If the plant has the necessary nutrients, the insect could develop to maturity producing more cage-induced negative information.

The obvious solution to this problem is a cage that will insure that the insect has had good opportunity to be in contact with the test plants, but also has the alternative of leaving any non-acceptable plants and searching for an acceptable plant. Figure 1 is a diagram of one possible design for a cage of this type. Being 20% open and 80% closed, this quasi-cage is already biased against the insect, but still another bias is proposed and that is to infest the test and control cages with insects that have been reared on their normal host and then starved before introduction into the test cages. Thus, if the insects have any predisposition to remain on the plants in the test cage, it will be sharpened by the preintroduction starvation.

This test has the advantage of exploring the reaction of the insect where the host plant is not available in the vicinity and the insect is exposed

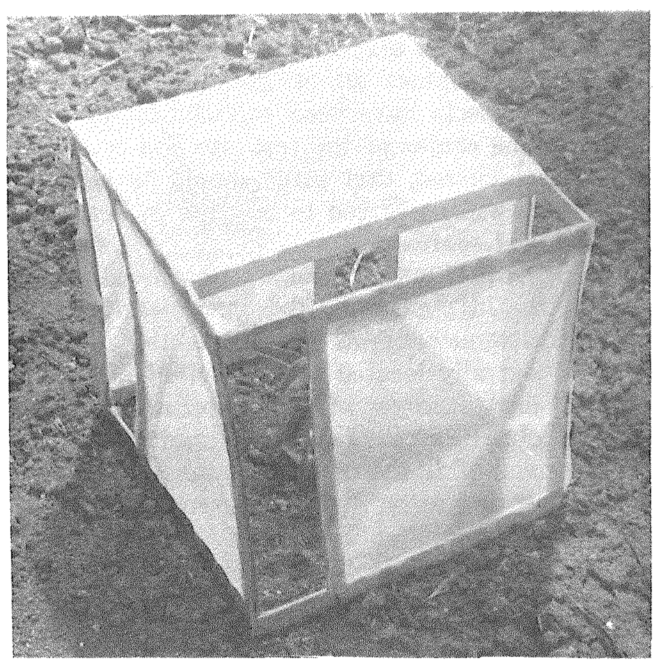

Figure 1. Quasi-Cage (80% closed, 20% open)

to but not restrained on test plants including those of economic value, while searching for a suitable host. If the test plants are unsuitable, the insect can leave in search of an acceptable host. If the insect is predisposed to select one or more of the quasi-caged plants as a host and feeds, oviposits, or perhaps matures, then there would be a high degree of probability that, that plant would be susceptible to attack by the insect being tested.

Maddox (1973) used the same principle satisfactorily in the laboratory during the testing of a thrips on alligatorweed (*Alternanthera philoxeroides* [Mart.]). The thrips has since been introduced and established in the U. S.

Non-cage tests

As a companion to this open cage trial, a trial without a cage is suggested that resembles the second one described by Harley (1968) and also a test used by Andres and Angalet (1963) in which they tested puncturevine weevils without a cage. They released *Microlarinus lareynii* (Jacquelin du-Val) and *Microlarinus lypriformis* (Wollaston) in commercial plantings of 9 crop plants, and in all cases the weevils abandoned the crop plants but remained in the puncturevine (*Tribulus terrestris* L.) control. This insect has since been released and become established in the U. S. and does little harm to crop plants.

The basis of the test described here is a mixed planting of test plants arranged symmetrically around the host plant (Figure 2). For the sake of illustration, a configuration with 6 test plants is presented here, but in practice the configuration is flexible as long as searching insects have the opportunity to contact all of the non-host plants. When the insects have been released on the host plants at the center of the test site and establishment has occurred, that plant should be killed so the insects will be forced to migrate in search of another food source.

Dr. B. D. Perkins of the Fort Lauderdale Location, ARS, USDA (personal communication) employed this same concept, i.e., he infested and then killed a centrally located host plant when testing a weevil on waterhyacinth (*Eichhornia crassipes* [Mart.] Solms), but the tests were conducted under cages instead of in the open. An alternate method of inducing migration would be overpopulation or making an inundative release of starved insects on the central host plant thus allowing population pressure to force migration to the nearby test plants.

The control for this experiment would be a planting of host plants in the same configuration, infested with the same number of insects but with the central plant removed or overpopulated at the same time the one in the test plot is treated. The insects used in the control should be marked. This will allow the investigator to determine if insects show up in the control other than those released there. Marking of all individuals in both tests and control is preferred, but sometimes the numbers involved make it prohibitive.

Discussion of tests

These 2 tests, while not adaptable to the testing of candidate insects in all orders, would probably be adequate for the majority of adult beetles, bugs, small moths, and insects with exophagous larvae. There is some question about the usefulness of either the quasi-cage or open test with nervous fast flying insects. Also, this system is not adaptable to endophagous insects because they cannot transfer from plant to plant by themselves and any mechanical transfer by the investigator would impart a totally unnatural variable into the trial, circumventing the thing we are trying to measure.

These tests will define, for the insects that may be used in them, the ability of that insect to select useful or socially important plants as hosts for feeding and oviposition under an artificially induced but real stress situation. If the insects do not accept the test plants but accept the control plants, this should be ample evidence of the safety of the proposed biocontrol agent.

ALTERNATIVE TESTS

Proving a candidate safe at a foreign site is a giant step, but not enough to permit release at home because there is the local flora to be concerned about in the country where the release is to be made, i.e., plants of special agricultural varieties, ornamental value, wild life cover and food value, etc. The current system is to bring the insects into quarantine, cage them with the plants to be tested and start generating questionable information, along with some that is useful. There are a couple of things that can be done to avoid this waste. Probably the most important is to get from the review board their list of important plants prior to starting the overseas tests. With this list of plants in hand, determine how many can be imported or grown for scientific purposes at the overseas site so the testing will have to be done in the country of release. Conversations with colleagues have brought up a couple of things to be considered that would permit field tests, which

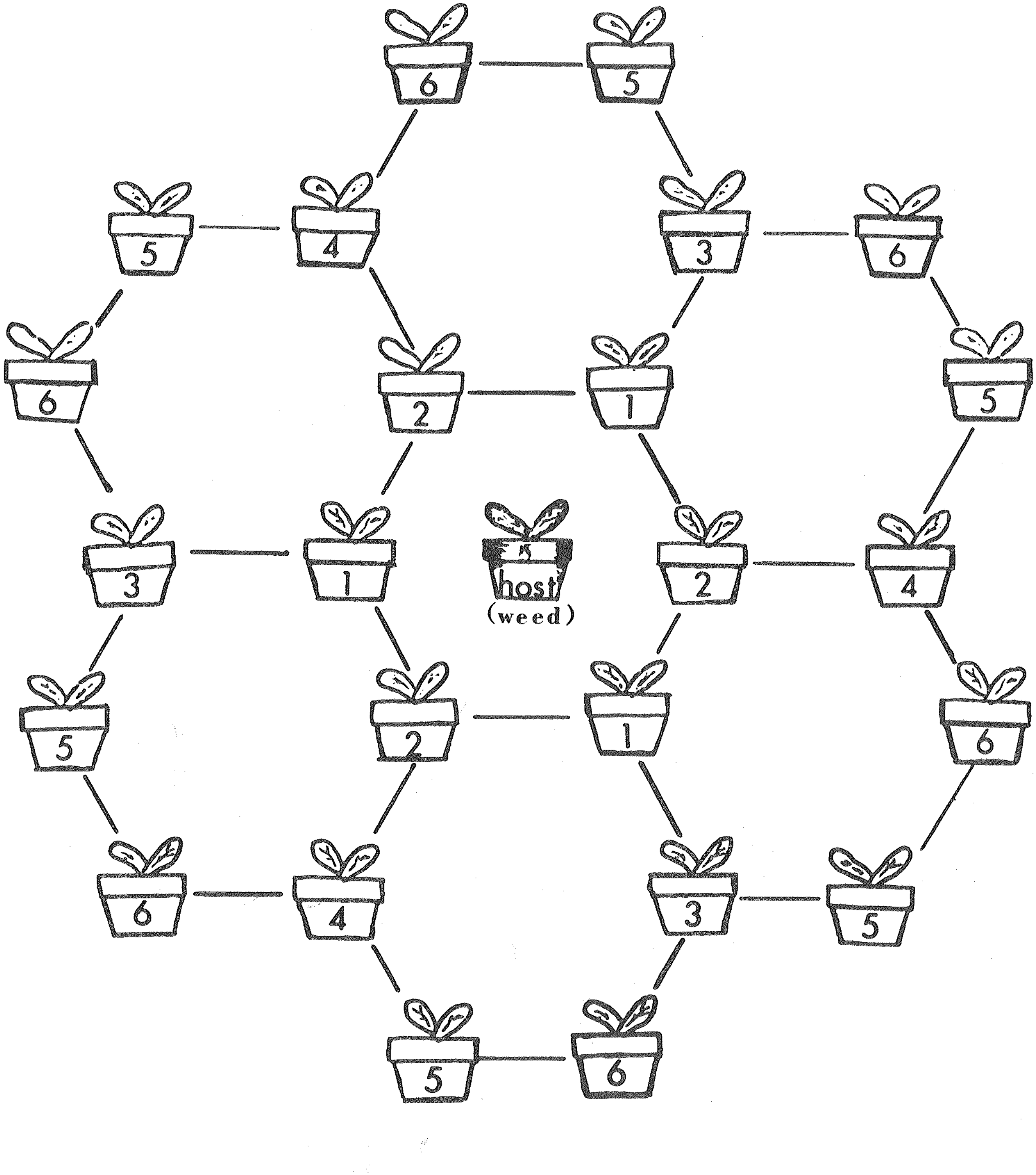

Figure 2. Configuration of planting for non-cage test using 6 test plants.

would produce positive information and eliminate the need for quarantine studies.

One is to simply sterilize the candidate insects by chemosterilants or radiation treatment and then conduct field trials at the domestic site where the insects would be released. A trial such as this would have few artifacts to be concerned about. Probably the main concern would be occult damage to the insect or alteration of the behavior by the sterilization process.

Another approach involving less complicated technology is the use of only unmated females or males for feeding studies in open cage and field tests. Use of these, of course, precludes oviposition studies and the use of and observation of immature forms.

An idea that could be considered for specialized circumstances in the countries that have severe winter climates is to design tests that are asynchronous with the seasons, i.e., start plants late and release insects on them late so that they would die of winter kill soon after the test data are taken. Admittedly this needs critical consideration before it is tried, and requires knowledge of the cold tolerance of the candidate insect.

Also there is a test that can be conducted in quarantine in cages that probably will produce useful information. This test requires time-lapse movies of insects caged separately with the test plants and the host plant controls. By this method, one would test host selection by photographing the movements of the caged insects at 5-minute intervals for 48 hours and by recording the amount of time they spent on the plant they were caged with. If in the control cage they spent the majority of the time on the plant and in the test cage the majority of the time trying to escape, this information could be interpreted to mean that the host selection mechanism of the insect does not recognize the test plant as a host. If continued for a while, no doubt the same old results would occur when the insects were hungry enough.

Some of these latter ideas may seem a bit bizarre, but we should be reminded that for the most part we are dealing with non-incriminated non-pest insects—that just need to have their status proven.

While being as imaginative as possible in our work, it must always be kept in mind that an unfortunate introduction works to no one's advantage.

REFERENCES

Andres, L. A. and G. W. Angalet. 1963. Notes on the ecology and host specifictiy of *Microlarinus lareynii* and *M. lypriformis* (Coleoptera: Curculionidae) and the biological control of puncture vine, *Tribulus terrestris*. J. Econ. Entomol. 56: 333-340.

Force, D. C. 1966. Reactions of the three-lined potato beetle, *Lema trilineata* (Coleoptera: Chrysomelidae), to its host and certain non-host plants. Ann. Entomol. Soc. Am. 59: 1112-1119.

Frick, K. E. and L. A. Andres. 1967. Host specificity of the ragwort seed fly. J. Econ. Entomol. 60: 457-463.

Frick, K. E. 1974. Biological Control of Weeds: Introduction, History, Theoretical and Practical Applications. Proceedings of the Summer Institute on Biological Control of Plant Insects and Diseases. F. G. Maxwell and F. A. Harris, editors. University Press of Mississippi, Jackson. 647 pp.

Harley, K. L. S. 1968. The suitability of *Octotoma scabripennis* Guer. and *Uroplata girardi* Pic. [Col. Chrysomelidae] for control of *Lantana* [Verbenaceae] in Australia. Bull. Entomol. Res. 58: 835-843.

Lawrence, G. M. H. 1951. Taxonomy of Vascular Plants. Eighth printing 1963. The Macmillan Co., New York. 823 pp.

Maddox, D. M. 1973. *Amynothrips andersoni* (Thysanoptera: Phaeothripidae), a thrips for the biological control of alligatorweed. 1. Host specificity studies. Environ. Entomol. 2: 30-37.

Wapshere, A. J. 1974. A strategy for evaluating the safety of organisms for biological weed control. Ann. Appl. Biol. 77: 201-211.

Williams, J. R. 1954. The biological control of weeds. Pages 85-98 *in* Report of the Sixth Commonwealth Entomol. Conference. Commonwealth Institute of Entomology, London.

Zwölfer, H. and P. Harris. 1971. Host specificity determination of insects for biological control of weeds. Annu. Rev. Entomol. 16: 159-178.