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UNITED STATES GOVERNMENT

Memorandum

TO : DAA/DS/FN, Tony Babb
THRU DS/PO, Robert Simpson

DATE: March 8, 1979

FROM : DS/AGR, for Dean F. Peterson

SUBJECT: Unsolicited Small Research Project Proposal, Montana State University "Regulation of Grasshopper and Locust Reproduction by Means of Plant Hormones" - $35,000

Problem: Your approval is required for an unsolicited small research project proposal (Attachment 1) from the University of Montana to investigate the possibility of using plant hormones to regulate grasshopper and locust reproduction. The research will be helpful in identifying possible methods for grasshopper control which will not involve the use of the persistent synthetic organic pesticides.

Discussion: The proposed research is the outgrowth of previous studies which have revealed statistically significant relationships between the temperature at which a host grass is grown and the fecundity and egg viability of an economically important rangeland grasshopper. The addition of different concentrations of two plant hormones, gibberellin A^3 and abscisic acid (already present in the host grass), produced highly significant reductions in fecundity and egg viability in the grasshoppers, not unlike those observed when the host grass was grown at different temperatures. Further, abscisic acid regulated fecundity and egg viability at concentrations ten times less than that required for a similar response with gibberellin A^3. The aim of this proposal is to develop standards for the use of abscisic acid as a grasshopper and locust control agent.

This project falls into one of the areas of research identified in A.I.D.'s Policy on Pesticide Support approved by the Administrator on June 6, 1978. In this Policy Statement A.I.D. stated that we would give special consideration in the Agency's research projects to the problems encountered by small farmers which might include among other things "development of non-chemical methods for pest control such as the use of sex attractants, anti-feeding compounds, juvenile hormones and micro-organisms pathogenic to pests."

Grasshoppers, including locusts, continue to be major pests in many parts of Africa and Asia and frequently are the cause of extensive crop damage in Latin America. For example, although drought conditions in the Sahel began to ease somewhat in 1973, and some food crops could again be grown in the more arid areas, severe pest outbreaks, particularly

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of grasshoppers, soon began to occur and, by 1974, heavy losses were being experienced. For example, farmers attempting to grow crops in flood recession areas of Mauritania, Mali and Senegal were forced to reseed two and sometimes three times, thus causing an estimated production loss of 30 to 60 percent. In Chad, Niger, Northern Cameron, Benin (formerly Dahomey), Togo, Upper Volta, Senegal and Mauritania, 75 to 80 percent of the millet crop was destroyed over several hundred thousand hectares. In Mali alone, 200,000 hectares, representing 16 percent of the total land under millet cultivation, suffered 80 to 100 percent losses. This represented a food crop loss of between 77,000 and 96,000 metric tons.

Furthermore, a number of the pesticides used for grasshopper control in these countries are the more persistent, broad spectrum pesticides, such as BHC and dieldrin, the registered uses of which have been cancelled by the USEPA because of their adverse environmental impacts. Hence there is an urgent need to develop more environmentally acceptable control measures for grasshoppers including locusts.

The proposed research will consist of three steps. First, biochemical determination of the titers of gibberellins and abscisic acid in the host grass at different periods in its growth and under different environmental conditions. Second, determination of the concentrations of abscisic acid which must be added to the host grass to regulate fecundity and egg viability. Third, testing the effects of various concentrations of abscisic acid added to their host plants on the reproduction and viability of representative species of other insect orders likely to be sympatric with grasshoppers and locusts.

The research will be conducted by Professors Visscher and Lewis of Montana State University who are eminently qualified to perform the research (see C.V.'s attached to the proposal).

The project proposal has been reviewed by four specialists within DSB:

Dr. Floyd Williams
Dr. Robert Jackson
Dr. James Erickson
Dr. Frederick Whittemore

The consensus of these specialists is that this is a good proposal with sound methodology. They caution however, that additional work on the economics and the possible environmental impacts of the use of abscisic acid for grasshopper and locust control would be required

should the proposed research confirm that abscisic acid does in fact regulate fecundity and egg viability in grasshoppers.

This latter aspect has been discussed with the investigator in the light of an EPA position paper dated December 4, 1978 on the "EPA Regulation of Biological Pesticides". That paper states that EPA proposes to publish draft guidelines for public comment specifying data requirements [illegible] registration of such products in July of 1979 with final guidelines to be promulgated in 1980. A copy of these draft guidelines, if they currently exist, will be furnished to the investigator with [illegible] to take these data requirements into account, insofar as possible, within the experimental design of the proposed research.

Recommendation: That you approve the allocation of $35,000 for the proposed research by signing below:

APPROVED: [signature]

DISAPPROVED: _____

DATE: 22.3.79

Attachments

Clearances:

DS/AGR/FCP:JMYohe [signature] Date: 3/8/79
DS/AGR/R:FWilliams (DRAFT) Date: 3/7/79
DS/AGR:MMozynski [signature] Date: 3/8/79
DS/PO:RRogers [signature] Date: 3/7/79
DS/PO/RES:MRechcigl [signature] Date 3/7/79

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AN UNSOLICITED PROPOSAL SUBMITTED TO THE

AGRICULTURAL AGENCY FOR INTERNATIONAL DEVELOPMENT

by

Montana State University, Bozeman, Montana
59717

Regulation of Grasshopper and Locust Reproduction by Means of Plant Hormones

Principal Investigator

Name:	Saralee Neumann Visscher
Title:	Professor of Entomology
Social Security No:	506 - 26- 6806
Department:	Department of Biology

Proposed Starting Date	Amount Requested	Duration
January, 1979	$35,000.00	One year

attachment 1

SUMMARY OF THE PROPOSAL

Investigations seeking intrinsic and extrinsic factors underlying dramatic fluctuations in numbers in wild populations of an economically important rangeland grasshopper, Aulocara elliotti (Thomas), revealed statistically significant relationships between the growth temperatures of the host grass, Agropyron smithii Rydb., and the reproductive success of this grasshopper. The addition of different concentrations of two plant hormones, gibberellin A^3 and abscisic acid, to this wheatgrass produced highly significant reductions in fecundity and egg viability when fed to A. elliotti, not unlike those observed when this host grass was grown at different temperatures. Further, abscisic acid regulated fecundity and egg viability at concentrations ten times less than required for a similar response with gibberellin A^3.

Since the quantities of these hormones present in the grass before hormone treatments are unknown, it is necessary to determine these titers in relation to different growth conditions before dose-response curves can be formulated for regulation of grasshopper reproduction.

The aim of this proposal is to develop standards for the use of abscisic acid as a grasshopper and locust control agent. This will be accomplished first by biochemical determination of the titers of gibberellins and abscisic acid in the host grass at different periods in its growth and with different environmental conditions, and second by determination of the concentrations of abscisic acid which then must be added to the host grass to regulate fecundity and egg viability, i.e., by developing dose-response curves for different conditions of the host grass and the grasshopper. Finally, representative species from other insect orders likely to be sympatric with grasshoppers and locusts will be tested with different concentrations of abscisic acid added to their host plants under defined conditions to determine the effects upon reproduction and viability.

PREVIOUS WORK, PERTINENT LITERATURE AND PROJECT RATIONALE

Grasshopper and locust outbreaks continue to cause major economic losses to agriculture despite world-wide control efforts. Increasing limitations of certain pesticides because of possible hazards to human health further complicate the problem of locust control. Recent research efforts have centered on developing biological methods for control which will provide ecologically acceptable pest management. Parasites and pathogens are being tested for their efficacy as control agents. Phytohormones which act as insect hormone mimics or analogues[1] are being studied for their ability to interfere with developmental processes, thereby preventing normal adult maturation. Host plant - insect interrelationships have been studied to find other compounds with biological activity. Recently, anti-juvenile hormones, the precocenes,[2] compounds which cause precocious metamorphosis in hemimetabolous insects, were isolated from plants. Other compounds have been discovered which act as feeding inhibitors in locusts,[3] suggesting yet another possible approach to management of these pests.

Over the past decade my research, sponsored by the Montana Agricultural Experiment Station, has been concerned with demonstration of the factors underlying dramatic fluctuations in population numbers in the big-headed grasshopper, Aulocara elliotti. This species is indigenous to the western United States, Canada, and northern Mexico and is ranked sixth among the economically important grasshoppers by the U.S.D.A. Following descriptive studies on the biology and embryonic development,[4,5] the effects of maternal environmental factors on reproductive success and embryonic viability were investigated. Maternal temperatures, density, photoperiod, and aging caused significant effects upon fecundity and embryonic development.[6] Subsequently, long-term studies were undertaken to determine whether there are significant changes in the fecundity and egg viability of geographically isolated wild

populations which could account for their population fluctuations. Fifth instar nymphs were collected annually from six populations and maintained throughout their adult lifespans in an insectary with similar rearing methods over eight consecutive summers. Each year, 10 to 15 single pairs of adults from each population were reared with both warm and cool temperature regimens, both fluctuating diurnally, under naturally shortening daylengths. Highly significant declines in fecundity and egg viability were observed in two of these populations over a four year period, while two other populations showed increases in those traits during the same years. Significant differences also occurred within single populations in different years.[7] Since the adults of all populations had been reared similarly, it was concluded that these reproductive differences probably arose, in part at least, from variable environmental factors acting on the nymphs before they were collected, or perhaps from maternal or genetic factors within the different populations.

Subsequently, experiments were conducted to test the effects of different rearing temperatures upon the fecundity of A. elliotti and concurrently, of the effects of temperature during the growth of the host plant as it might affect changes in reproduction when fed to the grasshopper. Western wheatgrass (Agropyron smithii), the primary host plant of A. elliotti, was grown in a warm environment and fed to grasshoppers reared at either warm or cool temperatures. Grass grown in the cool environment was fed to grasshoppers reared at both temperatures, and in addition, grass collected from a field site was fed to grasshoppers in both environments. Field temperatures fluctuated much more widely that either the warm or cool temperature environments during the rearing season. Highly significant effects of both the grasshopper rearing temperatures and host grass growth temperatures on fecundity and egg viability were found.[8] It appeared that qualitative

differences in the host grass were induced by changes in temperature which either stimulated or decreased egg production and viability. Analysis of total nitrogen in grasses grown at different temperatures showed no significant differences; field grass had less nitrogen than grass from either the warm or cool environments.

Because the synthesis and release of plant hormones can be affected by changes in temperature,[9] as well as other factors such as moisture and photoperiod, it seemed possible that these growth regulators might change sufficiently to be involved in the reproductive differences induced by growing temperatures of the host grasses. Search of the literature provided several reports concerning the effects of plant hormones upon insect fecundity and development. Delayed sexual maturation induced in the desert locust by feeding upon senescent vegetation was reversed when gibberellin A^3 was added to the diet[10]. Stimulatory effects of this hormone are known in Drosophila[11]. Conversely, gibberellin (GA^3) reduced the fecundity of the cotton leafworm after larval treatment[12], and adversely affected larval and pupal growth and fecundity of the bollworm[13].

Another plant hormone, abscisic acid (ABA), a compound with growth retarding function in plants, was tested with spruce budworm larvae in an artificial diet and on balsam trees without significant effects[14]. When ABA was fed to bean aphids on leaf discs, however, fecundity and rate of development were increased[15].

Such paradoxical functions of these hormones in insects, hormones which are generally antagonistic in plants, could perhaps be explained by the application of different concentrations to different species at different times in their ontogenies. What seems important, however, is that plant hormones can have significant effects upon insect reproduction at all. I suggest that plant hormones may have been utilized by insects to achieve

seasonal adaptation with their host plants, the growth stimulating hormones such as the gibberellins signalling the insects biochemically the appropriate time for growth and reproduction, and the growth inhibiting hormones, such as abscisic acid, providing the signal for cessation of reproduction. That abscisic acid acts to inhibit DNA synthesis in plants seemed supportive to such a hypothesis. The effect of ABA on animal metabolism was unknown.

Initial investigations to test the effects of these two plant hormones on the reproduction of A. elliotti were begun in 1977. Two concentrations of GA^3 and ABA were added to western wheatgrass by soaking and then watering the cut grass placed in the food vials.Treated grass was fed to adult grasshoppers over their entire lifespans; egg pods were collected daily, incubated at 25oC according to published methods[6], and the eggs fixed at 30 days of age and stored for examination. Highly signficant differences in both fecundity and egg viability were obtained with both hormones. Similar reproductive responses were achieved however, with concentrations of ABA ten times less than with those of GA^3 (See Figure 1). The inhibiting effect of ABA upon the embryonic metabolism was affirmed by immersing post-diapause eggs of A. elliotti in solutions of ABA in insect Ringer's solution in the presence of tritiated thymidine. Significantly lower incorporation was observed than in the controls,while GA^3 stimulated incorporation[16]. The inhibiting effects of ABA on insect reproduction and metabolism have not been previously shown, although suggestions of a weak juvenile hormone effect from exposure to ABA were observed[14,17].

In 1978 a second experiment was conducted in which ABA was added to western wheatgrass and fed to A. elliotti from the same wild population tested in 1977. In the second study, however, wheatgrass was grown in the insectary rather than in a greenhouse, and growth temperatures were those of the warm environment of the grasshoppers. Reduced fecundity and egg viability again were observed, but greater reduction occurred with increas-

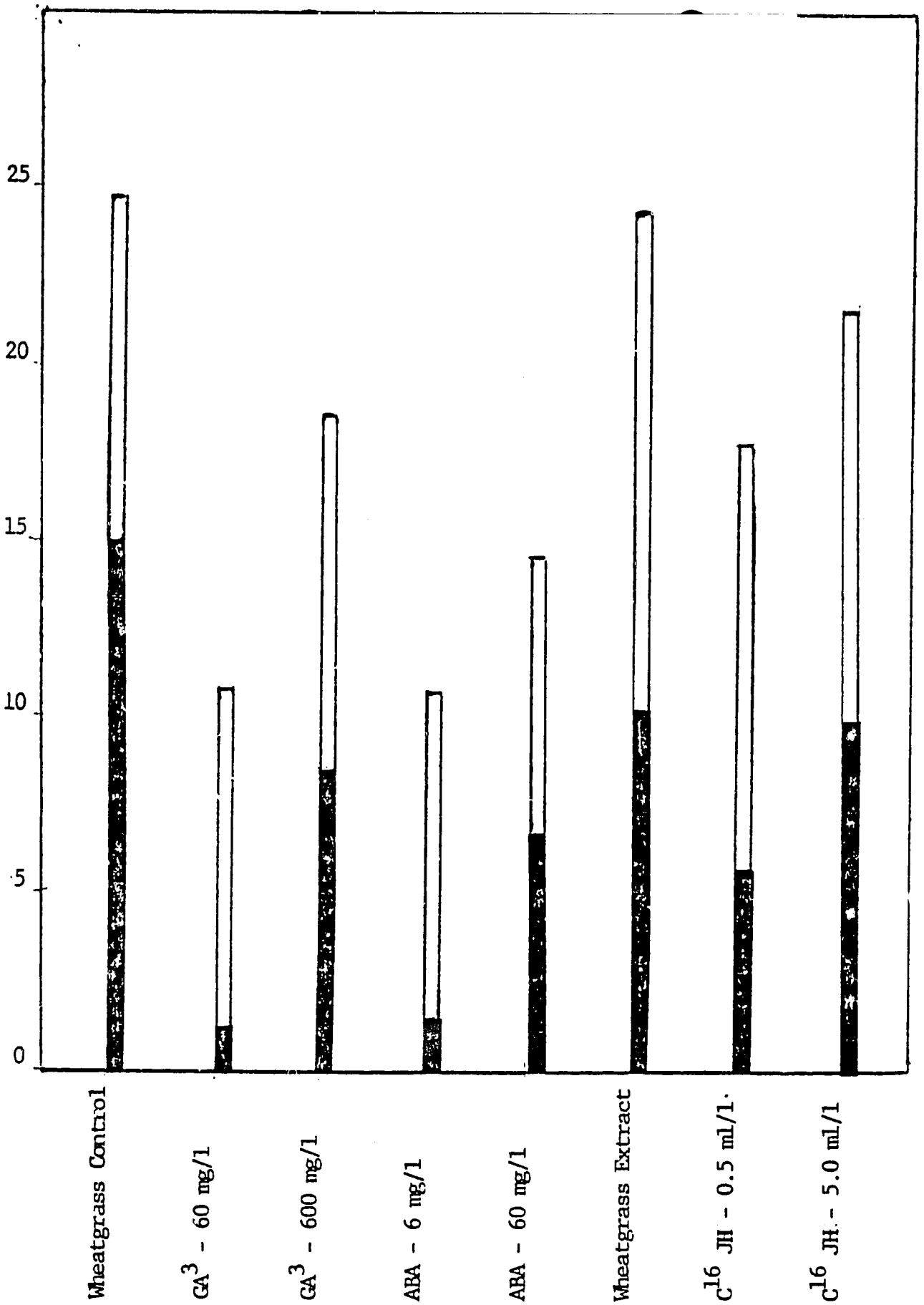

Figure I. Fecundity and Egg Viability of Aulocara elliotti fed Western Wheatgrass Treated with Plant and Insect Hormones.

ing concentrations at two of the doses tested. The response at a higher dose was not statistically different. It appears that substances, perhaps GA^3 and ABA, within the host plant may interact with the hormones added to them to alter the reproductive response of the insect.

Although it appears that ABA and GA^3 sprayed on the wheatgrass does not penetrate the surface cuticle, this possibility must be considered and the effects of these hormone treatments compared with untreated controls over the life cycle of the plant.

The conditions of synthesis, storage and release of GA^3 and ABA in western wheatgrass are unknown. It seems likely, however, that under different growing conditions, the titers of hormones will vary. Likewise, at different growth stages, changing titers of hormones would be expected. A relationship between GA^3, ABA and the terpenoid juvenile hormone of insects seems likely, since mevalonate, geranyl-geranyl pyrophosphate and/or farnesyl pyrophosphate may be common precursors. Research with radioactive compounds will be necessary to determine the site and mode of activity of ABA within the insect, but this aspect is beyond the scope of this project.

The immediate objectives of this proposal are concerned with obtaining information necessary to develop ABA as a safe and effective means of grasshopper and locust control.

OBJECTIVES

The objectives of this proposal are confined to three major goals.

1. To obtain biochemical data concerning the titers of gibberellin A^3 and abscisic acid present in the wheatgrass under different growth conditions, and to correlate these with concurrent reproductive success of the grasshoppers as both the grasses and grasshoppers change with time.

OBJECTIVES (continued)

2. To determine the concentrations of ABA which must be added to the wheatgrass, given the information obtained above, to curtail egg production and reduce egg viability to acceptable levels.

3. To test the effects of ABA on representative insects from other orders, such as Hymenoptera, Coleoptera, Lepidoptera, Neuroptera, Diptera, sympatric with the grasshoppers and on other Acrididae (Locusta migratoria).

REFERENCES CITED

1. Williams, C.M. and W.E. Robbins, 1968. BioScience,18:791-2;797-9.

2. Bowers, W.S., T. Ohta, J.S.Cleere, P.A. Marsella, 1976. Science,193: 542-547.

3. Bernays, E.A. and R.F. Chapman, 1977. Ecological Entomol.2: 1 - 18.

4. Van Horn, (Visscher)S.Neumann, 1963. PhD. Thesis, Montana State Univ. Bozeman, Montana. Pp. 1 - 172.

5. Van Horn, (Visscher)S.Neumann, 1966a. Jour. Morphol., 120: 83 -114.
Ibid 1966b. Jour. Morphol., 120:115 -134.

6. Visscher, S. Neumann, 1971. Ann. Entomol. Soc. Amer., 64:1057-1074.

7. Visscher, S. Neumann, 1977a. Japan Soc. Arthropodan Embryol.,Vol.13. Symp. at Gamagori, Japan. Pp. 9 - 17.

8. Visscher, S. Neumann, R. Lund and W. Whitmore. 1979. Environ. Entomol. In Press.

9. Meyer, B.S., D.B.Anderson, R.H. Rohuing and D.G. Fratianne, 1973. In: Introduction to Plant Physiology, D. Van Nostrand Co., New York.

10. Ellis, P.E., D.B. Carlisle and D.J. Osborne, 1965. Science, 149:546-547.

11. Alonso, C. 1971. Ent. exp. & appl., 14: 73 - 82.

12. Salama, H.S. and A.M. El-Sharaby, 1972. Experientia, 28: 413-414.

13. Guerra, A. A. 1970. J. Econ. Entomol., 63: 1518 - 1521.

14. Eidt, D.C. and C.H.A. Little, 1970. J. Econ. Entomol., 63: 1966 -1968.

15. Scheurer, S., 1976. In: The Host-Plant in Relation to Insect Behaviour and Reproduction. Pp. 255 - 259. T. Jermy, Ed. Plenum Press, New York.

16. Visscher, S. Neumann and S. Hapner, 1978. Unpublished data.

17. Williams, C.M., 1978. Personal communication.

EXPERIMENTAL PLAN, METHODS AND PROCEDURES

These items parallel the Objectives I - III outlined in this proposal.

I. Plant Culture.

To obtain biochemical information concerning the titers of ABA and GA_3 present in western wheatgrass grown under different temperature conditions and at different stages of growth, cultures of Agropyron smithii will be grown from seeds of a single strain in a greenhouse insectary with natural daylengths and diurnally fluctuating temperature regimens (warm, 24 - 30oC; cool, 18 - 24oC)and in a Percival controlled environment chamber with 12:12 hours daylength/darkness at cool temperatures (18 - 24oC). Twenty gram samples of wheatgrass will be collected at weekly intervals beginning April 1 until September 15, 1979 from each growth regimen and these processed for biochemical analyses according to methods described below. The concentrations of ABA and GA_3 present in grasses will be graphed in terms of the date the grass was taken and the temperature at which the grass was grown.

Insect Rearing

Concurrently, regimens of 20 pairs of Aulocara elliotti, collected as nymphs from a single wild population, will be fed throughout their reproductive lifespans on grasses from each of the growth regimens. Grasshoppers fed on these grasses will be considered to be "controls" and their reproduction will establish baseline fecundity and egg viability numbers for this experiment. Grasshoppers will be reared in the same greenhouse insectary as the grass in a warm environment (24 - 30oC) with natural daylengths. Eggs will be collected daily from pans at the bottom of every cage by sifting the soil therein, and egg pods then will be incubated vertically in plaster of Paris blocks for 30 days at 25oC. Methods for rearing A. elliotti, incubating and fixing eggs and evaluating embryonic development according to defined staging criteria are those developed for this species by the author[4,5,6]. At 30 days of age at 25oC, all eggs will be fixed in Bouins solution, rinsed and stored

in 70% ethanol. Subsequently embryonic viability will be determined using a Wild M-5 binocular dissecting microscope.

II. Additional groups of 20 pairs of adult A. elliotti will be fed throughout their lifespans on grasses from each of the growth regimens treated with solutions of ABA at concentrations of 6, 60 and 600 mg/l distilled water. Clipped grasses will be soaked four hours in treatment solutions, separated into bundles of 2-3 gm into food vials and watered with treatment solutions while grass is being fed to the grasshoppers. A similar method was used earlier and found to be effective in reducing the reproduction of A. elliotti (Figure 1). Data for fecundity and egg viability will be assessed for each feeding regimen and statistical analyses made of comparisons between control groups and those fed treated grasses, and between control regimens grown at different temperatures. Such comparisons should reveal the conditions of growth which support the highest and lowest reproductive rates, and what concentrations of ABA must be added to grasses grown under different conditions to achieve reproductive regulation. When fecundity and egg viability data are then related to biochemical data for titers of ABA and GA_3 present in the grass at given time intervals, dose-response curves can be formulated with some understanding of anticipated interaction between the endogenous and applied hormone concentrations.

III. To determine the effects of ABA treatment on insects likely to be sympatric with grasshoppers, tests will be made on a number of representative insect species from different orders of Insecta. Two other species of Acrididae also will be tested for comparison of dose-response effects with those of A.elliotti. The species proposed for testing and the media proposed for their maintenance:

Diptera - Drosophila melanogaster - fruit fly - artificial medium

Mayetiola destructor - Hessian fly - wheat plants in vitro

Homoptera - Rhopalosiphum padi - oat birdcherry aphid - barley plants

Hymenoptera- Cephus cinctus - wheat stem sawfly - wheat plants

Coleoptera - Tenebrio molitor - mealworm - bran and potato

Coleoptera - Hypera brunneipennis (postica)- alfalfa weevil - alfalfa plants

Lepidoptera - Heliothis zea - corn earworm - artificial diet

Orthoptera - Melanoplus sanguinipes - Mexican grasshopper - barley plants, bran

Locusta migratoria - African migratory locust - grasses,lettuce

Three doses of ABA will be applied to the host plant or incorporated into the artificial medium and fed to immature stages and/or to the adults, depending upon the behavior of the species. Developmental rates, fecundity and egg viability will be assessed and compared with that of the untreated controls.

I. Analytical Biochemical Methods

Approximately 20 gm samples of wheatgrass will be taken at weekly intervals as they are being cut for feeding to grasshoppers and immediately frozen (-20oC) in preparation ofr analysis. Methods of Wills et al., 1976[18] and Garrod,1974[19] will be used.

Abscisic acid

Grass samples will be ground and blended and twice extracted in methanol containing 0.2% butylated hydroxytoluene. Extracts will be reduced in volume in a rotary evaporator at 40oC and the concentrate will be taken up in 0.5M Pi buffer (pH 8.2). The ABA fraction will be further concentrated by partitioning against several solvents as reported by Zeevaart, 1971[20]. Recovery of "bound" ABA will also utilize this method. The final ethyl acetate fraction will be further purified by preparative thin layer chromatography on silica gel plates using a solvent mixture of butanol: propanol: NH_4OH: H_2O (2:6:1:2 v/v). The ABA zone on the plates will be located by markers of standard ABA visualized under UV light (254 nm) with aluminum foil protecting the analytical portion of the plate. The ABA zone will be eluted by water saturated with ethyl acetate. The ABA will be methylated by diazomethane[21] and assayed by gas liquid chromatography using a FID and a glass column containing 2% QF-1 on Chromosorb at a temperature of 187o. Authentic ABA will be used to determine retention times and the identity of the natural ABA will be confirmed by mass spectographic analysis of collected fractions.

Gibberellins

The samples of grass will be macerated and extracted 48 hrs with absolute ethanol, followed by a second extraction,48 hrs, with 70% ethanol. The filtrates will be reduced to dryness under vacuum and taken up in 5% sodium bicarbonate at pH 8.0. This solution will be extracted three times with equal volumes of ethyl acetate. The ethyl acetate fraction will then be purified by passage through a column of Polyclar AT and the GA eluted with 0.1 M phosphate buffer at pH 8.0. The eluate will be adjusted to pH 2.5 and the GA recovered by partitioning with ethyl acetate. This fraction will be concentrated and chromatographed on silica gel plates (0.25 mm) using the solvent system of MacMillan and Suter,[22] 1963. The appropriate band of silica gel will be eluted with H_2O saturated with ethyl acetate. The eluates will be bioassayed by the barley endosperm test[23] or with Grand Rapids lettuce seeds in the dark. Alternatively, the eluates will be assayed by GLC using the trimethylsilylether-methyl ester derivative of GA as described by Schneider et al,[24] 1975.

REFERENCES CITED FOR METHODS

18. Wills, R.B.H., K.J. Scott and M.J. Franklin, 1976. Phytochem.,15:1817.

19. Garrod, J.F., 1974. J. Exp. Botany, 25: 945.

20. Zeevaart, J.A.D., 1971. Plant Physiol., 47: 421.

21. Schlenk, H. and J.L. Gellerman, 1960. Anal. Chem., 32: 1412.

22. MacMillan, J. and P.J. Suter, 1963. Nature, 197: 790.

23. Nichols, P.B. and L.G.Paleg, 1963. Nature, 199: 823.

24. Schneider, G., J. Siegfried, and S.J. Gunther, 1975. J. Chromat.,109:409.

FACILITIES AVAILABLE FOR THIS PROJECT

Space. Two 16'X 16' laboratories in Cooley Research Building.

Two temperature controlled rearing rooms in an insectary; one 14' X 16'; one 28' X 16'.

Laboratory equipment

One Percival controlled environment chamber - Model PGC-975.

Two temperature controlled incubators (Precision and Fisher) for incubating eggs under constant temperature conditions.

Sub-zero freezer for frozen grass samples.

300 clear plastic cages ($8 each)(11" X8½" plastic cylinders on 9" aluminum cake pans) for rearing insects.

Two M-5 Wild binocular dissecting microscopes, one with photography attachments; for determining egg viability.

F + M gas chromatograph and recording attachment.

Varian CH-5 mass spectrometer on a rental basis (Chemistry Dept.)

Equipment for thin layer chromatographic separations.

Collecting - Travel

GMC 4X4 3/4Ton Truck from the State Motor Pool - rental 17¢ per mile.

PERSONNEL

Principal Investigator: Saralee Neumann Visscher (formerly Van Horn)

Education: B.A. (Biology) University of Montana, Missoula, 1949
M.S. (Entomology) Montana State Univ.,Bozeman, 1958
Ph.D.(Entomology) Montana State Univ.,Bozeman, 1963
Pre-doctoral, University of Kansas, Lawrence, 1958-59
Post-doctoral, University of Virginia,Charlottesville,1965-66
N.S.F. Institutes, Vanderbilt Univ.,Nashville, Histochemistry,1966; University of Washington,Friday Harbor,Comp.Invertebrate Embryology, 1970.

Experience: Research Assistant, Dept. Zoology-Entomology,Mont.State Univ.1958
Instructor,½ time, University of Kansas, 1958-59.
Graduate Research Asst. Montana State, 1959-60;
Asst. Professor of Entomology, Montana State, 1962-67
Assoc.Professor, Montana State, 1967-72
Full Professor, Montana State, 1972 - present

Awards and Honors:
N.S.F. Pre-doctoral Fellowship, Montana State, 1960-61
N.I.H. Post-doctoral Fellowship,Univ. Virginia,1965-66
N.S.F. Travel Award, XIIth Intern.Congress of Entomol.,London, 1964
N.I.H. Research Grants, 1962-1968 with Dr. James H. Pepper
N.S.F. Grant for the Rocky Mt.Regional Conference Symp.of the Soc. for Developmental Biology, 1971

PERSONNEL (continued)

S. Neumann Visscher- Awards and Honors

N.S.F.- U.S.-Japan International Cooperative Research Grant with Dr. Hiroshi Ando, 1977 - 1979

Organizer - Symposium on Insect Embryology, XIVth Intern. Congress of Entomology, Washington,D.C. 1976

President-elect, Montana Academy of Sciences

Past-President, Montana State Univ. Chapter, Sigma Xi

Invited lectures at universities in the U.S.,Canada,Japan

Publications: Ph.D. Thesis. The Embryogenesis and Embryonic Variability of Aulocara elliotti (Thomas)(Orthoptera,Acrididae),Montana State University, Bozeman, 1963. Pp. 172. (Van Horn)

1966a. (Van Horn). Studies on the embryogenesis of Aulocara elliotti (Thomas)(Orthoptera,Acrididae).I. External morphogenesis. Jour. Morphol. 120: 83 - 114.

1966b. (Van Horn). Studies on the embryogenesis of Aulocara elliotti (Thomas)(Orthoptera,Acrididae). II.Developmental variability and the effects of maternal age and environment. Jour. Morphol.,120:115 - 134.

1968 (Van Horn). Gland volume changes during embryogenesis of Aulocara elliotti (Thomas)(Orthoptera,Acrididae).Proc. Mont.Acad.Sci.,28: 2pp

1971.(Visscher) Studies on the embryogenesis of Aulocara elliotti (Thomas)(Orthoptera, Acrididae). III. Influence of the maternal environment and aging on development of the progeny. Ann. Ent. Soc. Amer., 64: 1057 - 1074.

1972. Analysis of the embryonic diapause of Aulocara elliotti(Thomas) Histological, quantitiative and experimental. Proc.XIVth Intern.Congress of Entomology, Canberra, Aust. P. 138.

1972. M.H.Wessel and S.N.Visscher. Nuclear migration during blastoderm formation in Aulocara elliotti(Thomas).Proc.Mont.Acad. Sci., 32: 15-17.

1972. Peripheral ganglia in orthopteroid insects. Proc. Mont.Acad.Sci., 32:14.

1976. The embryonic diapause of Aulocara elliotti: Histological and morphometric changes during diapause development and following experimental termination with juvenile hormone analogue.Cell and Tis.Res.,174: 433 - 452.

1977a Experiments on the embryonic physiology of the big headed grasshopper, Aulocara elliotti (Thoams): Orthoptera, Acrdidae. Japan Soc. Arthropodan Embryology, Vol. 13: 9 - 17

1977b The developing grasshopper egg: a sensitive indicator of the maternal physiology and future population numbers in Aulocara elliotti (Orthoptera: Acrididae). VIIIth Intern. Congr. Intern. Soc. for Developmental Biology, Tokyo, P.61.

1978. M. Gaudet and S.N.Visscher. The effects of photoperiod on wing formation in the aphid Rhopalosiphon padi L. Proc. Mont.Acad.Sci. In Press.

Publications (continued)

In press. Visscher, S.N., R. Lund and W. Whitmore. Host plant growth temperatures and insect rearing temperatures influence reproduction in the grasshopper Aulocara elliotti (Orthoptera: Acrididae). Environmental Entomology.

In press. Embryonic Diapause: Morphological and Physiological Perspectives. Chapter 8, in "Aspects of the Embryonic Physiology of Insects", S.N.Visscher, Ed. Big Sky Press, Montana State University, Bozeman, Montana 260 pp.

In preparation. Regulation of grasshopper fecundity, egg viability and female longevity by antagonistic plant hormones.

In preparation. Estimating population dynamics in a rangeland grasshopper by means of reproductive and developmental parameters.

Other Personnel

Co-Investigator: Roger W. Lewis

Education: B.A. (Chemistry and Zoology) Pomona College, Pomona,Calif. 1948
M.S. (Biology) University of Southern California,Los Angeles,1961
Ph.D. (Marine Biology) University of California,San Diego, 1965

Experience: Assistant Professor, Institute of Artic Research, University of Alaska, Fairbanks 1965-67
Research Fellow, D.S.I.R. Food Chemistry Division, Wellington, New Zealand, 1967-68
Research Fellow, C.S.I.R. Division of Food Research, North Hyde, New South Wales, Australia, 1968 - 69
Senior Research Scientist, D.S.I.R. Applied Biochemistry Division, Palmerston North, New Zealand, 1969-73
Senior Research Scientist,University of Otago Medical School, Dunedin, New Zealand, 1973 - 75
Adjunct Associate Professor, Montana State University, 1975-78
Chief Administrator, Chest Clinic, Dunedin Public Hospital, Dunedin, New Zealand

Publications: (List ~~to be provided~~ attached)

Dr. Lewis is a native of Montana, born at Wolf Point. He has more than twenty research publications in the area of lipid biochemistry, and has published two in the past two years from the Biology Department at Montana State University while on leave to the United States. Because his research has dealt with lipids in the mevalonate pathway, in particular, squalene, a precursor of geranyl and farnesyl phosphate, Dr. Lewis' background is especially well suited for the biochemical problems connected with this project.

Publications - R.W.Lewis

1962. Temperature and pressure effects on the fatty acids of some marine ectotherms. Comp. Biochem. Physiol.,6: 75.

1966. Studies of the glyceryl ethers of the stomach oil of Leach's petrel Oceanodroma leucorhoa (Viellot). Comp. Biochem. Physiol.,19: 363.

1967. Fatty acid composition of some marine animals from various depths. J. Fish. Res. Bd. Canada, 24: 1101.

1967. The vitamin A content of polar bear liver: range and variability. Comp. Biochem. and Physiol., 22: 923 (Lewis, R.W. and J.W. Lentfer)

1969. The fatty acid composition of arctic marine phytoplankton and zooplankton with special reference to minor fatty acids. Limnol.Oceanog.,14:35.

1969. Studies on the stomach oils of marine animals. I. Oils of the black shark Dalatias licha (Bonnaterre). Comp. Biochem. Physiol.,31: 715.

1969. Studies on the stomach oils of marine animals. II. Oils of some procellariiform birds. Comp. Biochem. Physiol.,31: 725.

1969. Distribution of dietary squalene in a wandering albatross, Diomedea exultans. Nature, 224: 1220.

1970. Densities of three calsses of marine lipids in relation to their possible role as hydrostatic agents. Lipids, 5: 151.

1970. Fish cutaneous mucus: a new source of skin surface lipids. Lipids,5: 947.

1971. Lipid composition of human bronchial mucus. Lipids, 6: 859.

1971. Squalene distribution in fish with normal and pathologically fatty livers. Int. J. Biochem., 2: 609.

1972. Squalene content of plant tissues. Phytochem., 11: 417.

1973. Vitamin A: Occurrence and distribution in fractionated mucus. Lipids, 8: 321.

1975. The squalene content of human atheromatous plaques: A re-examination. Atheroschlerosis, 22: 637.

1976. Mucus globule membrane: A hypothesis concerning its role in determining the viscosity of mucus. J. Theoret. Biol., 61: 21.

1976. The effect of alimentation on human serum squalene levels. Lipids,11: 430.

1978. The biochemical basis of cystic fibrosis: An hypothesis based upon the polyelectrolytes of mucus. Texas Rep. Biol. & Med., 36: 33.

REVISED BUDGET

Personnel

Saralee Neumann Visscher, Principal Investigator, 20% time; No Cost	
Roger W. Lewis, Co-Investigator, 100% time	$18.000.00
Fringe Benefits (14%)	2,520.00
Laboratory Assistant, B.S., 50% time; graduate student	4,800.00
Fringe Benefits (1%)	48.00
Subtotal	$25,368.00

Supplies

Plant hormones - ABA for feeding tests (Sigma Chem., $57/g)	3,250.00
GA_3 for biochemical standard	50.00
Chemicals for insect diets, solvents, prepared diets	350.00
Glassware and laboratory supplies	350.00

Travel and Per Diem

Rental of 4-wheel drive for collecting insects	400.00
Tourist class air fare, Bozeman to Kingston, Ontario, return, and per diem to conduct locust tests	1000.00
Air fare and per diem to Davis, Calif. to present findings at the 2nd Intern. Symposium on Invertebrate Reproduction	500.00
Subtotal	$5,900.00
Total Direct Costs	
Indirect Costs - 57.5% of salaries, wages and benefits	$14,587.00
Total	$45,855.00
Less: Montana State University Contribution	($10,855.00)
TOTAL AMOUNT REQUESTED	$35,000.00

Administrative Approvals:

Saralee N. Visscher 1/15/79

Principal Investigator Date

1/17/79

Budget Review, MSU Grants and Contracts Office Date

BUDGET JUSTIFICATION

Salaries and Wages.

The salary requested for Dr. R.W. Lewis is at the level set by the Montana State University salary schedule for senior adjunct research personnel. A half-time laboratory assistant is requested to help with rearing insects and maintaining egg cultures. Seven-day-a-week maintainance is necessary to obtain accurate data on fecundity and egg viability.

Travel.

Dr. Gerald Wyatt, insect physiologist-biochemist now of Kingston, Ontario, Canada, has been working with the plague locust, Locusta migratoria in his laboratory at Kingston. It is prohibited to import this species into the United States and therefore, it cannot be used here as an experimental animal. Dr. Wyatt has discussed with me briefly when we met at the Houston meetings of the Entomological Society the possibility of coming to Kingston in the next year to use this insect for testing. Travel and per diem to Kingston have been requested for this purpose.

Chemicals and Supplies.

The largest portion of the $4000.00 requested for chemicals and supplies will be used to purchase ABA for testing from Sigma Chemical Corp. Additionally solvents for biochemical analyses and some laboratory glassware will be required.

CURRENT RESEARCH SUPPORT

Currently I hold a National Science Foundation U.S.-Japan Cooperative Research Grant with Dr. Hiroshi Ando of the University of Tsukuba, Sugadaira, Japan, for a study of the comparative embryology of a primitive orthopteroid insect belonging to the Grylloblattidae, a species inhabiting only the mountains of Japan and western United States, and thought to be ancestral to

Current Research Support - continued

modern grasshopper types. The funds remaining in this project are approximately $2000 and these will have been encumbered by July, 1979.

SOCIOECONOMIC IMPACT OF THE PROPOSED PROJECT

Diminishing food and energy resources are ranked among the most imminent crises faced by man. Achieving safe and effective control of insect pests of agriculture is,therefore, of primary importance. One promising approach to this problem is by gaining better understanding of naturally occurring regulative mechanisms of insect control, such as those evolved by plants, and utilizing them as control agents.

The data obtained thus far indicates that the plant hormone, abscisic acid, effectively sterilizes grasshoppers at very low concentrations when applied to host plants. Further, abscisic acid is present in a wide variety of foods eaten by man,and has recently been patented as a human vitamin (U.S. Patent #3958025 issued to Virginia W-C Livingston). Tests with laboratory mice indicated no adverse effects from feeding ABA to mice at doses equal to 10% of their body weights.

Experiments to establish the effectsof ABA on growing plants must be conducted. It apparently does not penetrate the cuticle of wheatgrass, and has been said to have poor penetrating qualities. No adverse effects of foliar application, therefore, are anticipated, but data for individual plant species must be obtained before a reliable estimate can be made.