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BIOLOGICAL ACTIVITY OF THE N-ARYLPHTHALAMIC ACIDS
WITH SPECIAL REFERENCE TO FLOWERING OF
LYCOPERSICUM ESCULENTUM (MILL.)

BY

JOHN THOMAS WADDINGTON

A THESIS

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TITLE

BIOLOGICAL ACTIVITY OF THE N-ARYLPHTHALAMIC ACIDS WITH SPECIAL REFERENCE

TO FLOWERING OF LYCOPERSICUM ESCULENTUM (MILL.)

BY

John Thomas Waddington

APPROVED

DATE

J. M. Daly

June 1, 1962

J. F. Davidson

June 1, 1962

R. C. Lommasson

June 1, 1962

John H. Pazur

June 1, 1962

J. O. Young

June 1, 1962

SUPERVISORY COMMITTEE

GRADUATE COLLEGE

UNIVERSITY OF NEBRASKA

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DR. FREDERICK G. TEUBNER
WHO WILL EVER LIVE IN THE MEMORY OF HIS STUDENTS.

PREVIEW

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INTRODUCTION

THE EFFECTS OF SYNTHETIC GROWTH REGULATORS ON FLOWERING OF THE TOMATO HAVE BEEN EXTENSIVELY STUDIED IN RECENT YEARS. HOWEVER, ONLY THREE TYPES OF SYNTHETIC GROWTH REGULATORS, 2,3,5 TRIIODOBENZOIC ACID, α -(2-NAPHTHOXY) PHENYLACETIC ACID AND THE N-ARYLPHTHALAMIC ACIDS HAVE BEEN CLEARLY SHOWN TO STIMULATE TOMATO FLOWER FORMATION.

ALTHOUGH IT IS NOT CLEAR HOW THE N-ARYLPHTHALAMIC ACIDS REGULATE FLOWERING, THIS RESPONSE IS APPARENTLY RELATED TO THEIR ACTIVITY AS AUXINS. TEUBNER ET AL. (54) HAVE EXAMINED THE EFFECTS OF SUBSTITUTION IN THE PHENYL RING ON THE BIOLOGICAL ACTIVITY OF THESE COMPOUNDS. HOWEVER, THE EFFECTS OF MODIFICATION IN THE STRUCTURE OF THE SIDE CHAINS OF THESE COMPOUNDS ON BIOLOGICAL ACTIVITY HAVE NOT BEEN EVALUATED. A VARIETY OF STRUCTURAL MODIFICATIONS ARE POSSIBLE IN THE SIDE CHAIN OF THESE COMPOUNDS. EVALUATION OF THE EFFECT OF THESE MODIFICATIONS ON BIOLOGICAL ACTIVITY WOULD CONTRIBUTE TO KNOWLEDGE OF THE STRUCTURAL REQUIREMENTS AND ORIENTATION NECESSARY FOR ACTIVITY NOT ONLY OF THE N-ARYLPHTHALAMIC ACIDS, BUT OF AUXINS IN GENERAL.

THE LOSS OF BIOLOGICAL ACTIVITY WITH AGED SOLUTIONS OF THESE COMPOUNDS HAS BEEN ATTRIBUTED TO EITHER HYDROLYSIS OF THE AMIDE BOND OR AN INTRAMOLECULAR REARRANGEMENT OF THE MOLECULE (46). HOWEVER, MOST OF THE EVIDENCE IS IN-

CONCLUSIVE AND CONTRADICTORY RESULTS HAVE BEEN REPORTED BY DIFFERENT WORKERS.

THE PRACTICAL USE OF THE N-ARYLPHTHALAMIC ACIDS IN THE GREENHOUSE GROWING OF TOMATOES HAS BEEN ESTABLISHED BY WITTWER AND TEUBNER (63). N-M-TOLYLPHTHALAMIC ACID WHEN APPLIED TO TOMATO PLANTS AT THE "2-3 LEAF" STAGE AT A CONCENTRATION OF 200 PPM SIGNIFICANTLY INCREASED THE NUMBER OF FLOWERS FORMED IN THE FIRST TWO INFLORESCENCES OF THE PLANT. THIS INCREASE IN FLOWER NUMBER RESULTED IN A LARGER NUMBER OF FRUITS PER CLUSTER AND PER PLANT.

THESE FINDINGS WERE FELT TO BE OF POTENTIAL VALUE IN THE ADAPTATION OF FIELD GROWN TOMATOES TO MECHANICAL HARVESTING. SINCE THE PLANTS ARE DESTROYED DURING THE MECHANICAL HARVESTING OPERATION, ONLY ONE HARVEST PER PLANT IS POSSIBLE. THIS NECESSITATES THE "CONCENTRATION" OF YIELDS INTO A LARGE SINGLE HARVEST RATHER THAN THE CUSTOM-ARY 5 OR 6 SMALL HARVESTS NOW OBTAINED WITH HAND HARVEST-ING OPERATIONS. THE USE OF THE ARYLPHTHALAMIC ACIDS TO INCREASE FLOWER NUMBER COULD RESULT IN INCREASED YIELDS OF A SINGLE HARVEST OF FRUIT.

LITERATURE REVIEW

FLOWER FORMATION IN THE TOMATO

THE TOMATO PLANT, LYCOPERSICUM ESCULENTUM, MILL., HAS BEEN DESCRIBED AS A SYMPODIUM (59). GRAY (16) DEFINES A SYMPODIUM AS A STEM HAVING A SIMPLE AXIS, TERMINATED BY A FLOWER OR INFLORESCENCE, IN WHICH THE BUD IN THE AXIL OF THE LEAF SUBTENDING THE INFLORESCENCE DEVELOPS INTO A NEW AXIS OR BRANCH. THE VIGOROUS GROWTH OF THIS SYMPODIAL SHOOT FORCES THE ADJACENT INFLORESCENCE INTO A LATERAL POSITION AND THEREBY ASSUMES A DOMINANT ROLE. THE SYMPODIAL SHOOT BEARS ITS LEAVES AND TERMINATES, LIKE ITS PREDECESSOR, WITH AN INFLORESCENCE. A SECOND SYMPODIAL SHOOT THEN DEVELOPS FROM AN AUXILLARY BUD. TOMATO CULTIVARS IN WHICH THIS PATTERN OF DEVELOPMENT IS REPEATED INDEFINITELY ARE REFERRED TO AS INDETERMINATE TYPES. A SECOND PLANT TYPE RESULTS WHEN DEVELOPMENT OF THE SYMPODIAL SHOOT IS SUPPRESSED AFTER FORMATION OF ONE TO THREE INFLORESCENCES. THE LAST INFLORESCENCE REMAINS IN A TERMINAL POSITION. THIS IS GENERALLY ACCOMPANIED BY SUBSEQUENT VEGETATIVE GROWTH FROM OTHER LATERAL BUDS WHICH ALSO PRODUCE TWO OR THREE INFLORESCENCES BEFORE TERMINATION. TOMATO CULTIVARS HAVING THIS SECOND PATTERN OF DEVELOPMENT ARE CONSIDERED DETERMINATE TYPES.

THERE IS SOME DISAGREEMENT IN THE LITERATURE AS TO THE DESCRIPTION OF THE TOMATO INFLORESCENCE. BOTH COOPER (9) AND SMITH (48) DESCRIBE THE TOMATO INFLORESCENCE AS A RACEMOSE CYME, WHILE BOUQUET (6) STATES THAT THE SIMPLE

RACEME IS MORE COMMON IN A MIXED POPULATION WITH THE RACEMOSE CYME HAVING DICHOTOMOUS BRANCHING OCCURRING LESS FREQUENTLY. CRANE (10) IN AN EXTENSIVE STUDY ON THE HEREDITY OF TYPES OF TOMATO INFLORESCENCES FOUND THAT THE SIMPLE RACEME IS DUE TO A SINGLE DOMINANT GENE. BOUQUET FOUND THIS CHARACTER OCCURED IN ALL CULTIVARS STUDIED WITH THE EXCEPTION OF EARLIANA. DOMINANCE OF THE SIMPLE RACEME CHARACTER MAY BE ALTERED BY SEVERAL ENVIRONMENTAL FACTORS SUCH AS LIGHT, TEMPERATURE AND NUTRITION SO THAT TWO-, THREE-, AND MULTI-BRANCHED INFLORESCENCES ARE PRODUCED (8, 35, 52, 61, 62, 64).

THE EFFECT OF LOW TEMPERATURE DURING SEEDLING STAGES ON SUBSEQUENT FLOWER NUMBERS AND FLORAL INITIATION HAS BEEN EXTENSIVELY STUDIED AND IS NOW THE BASIS FOR CERTAIN CULTURAL PRACTICES IN THE GREENHOUSE PRODUCTION OF TOMATOES (61, 62, 63, 64). WENT (59) TERMS THIS RESPONSE "THERMOPERIODICITY" IN WHICH A DIURNAL FLUCTUATION OF TEMPERATURE WITH A NIGHT TEMPERATURE OF 18°C RESULTS IN LARGER FLOWER NUMBERS IN FLOWER CLUSTERS BEING INITIATED AT THE TIME OF THE LOW TEMPERATURE TREATMENT. HOFFMAN (19) IN STUDIES UNDER GREENHOUSE CONDITIONS FOUND A NIGHT TEMPERATURE OF 15 TO 16°C TO BE OPTIMUM WITH A DAY TEMPERATURE OF 21 TO 24°C .

LEWIS (35) HAS SHOWN THAT THE TEMPERATURE SENSITIVE PERIOD FOR THE FIRST INFLORESCENCE IN THE TOMATO OCCURS BETWEEN THE 8TH AND 12TH DAYS AFTER COTYLEDON EXPANSION.

EXPOSURE OF SEEDLINGS TO 13°C DURING THIS PERIOD RESULTS IN SIGNIFICANT INCREASES IN THE NUMBER OF FLOWERS FORMED IN THE FIRST INFLORESCENCE.

WITTWER AND TEUBNER (61, 62, 64) ALSO FOUND THAT THE NUMBER OF NODES SUBTENDING THE FIRST INFLORESCENCE WAS DECREASED WITH COLD TREATMENTS OF 10 TO 13°C IN CONJUNCTION WITH AN INCREASE IN FLOWER NUMBERS AS COMPARED WITH PLANTS GROWN AT 18 TO 21°C . THEY ALSO CONCLUDED THAT THE RESPONSE TO COLD TREATMENT IS AN ACCUMULATIVE EFFECT RATHER THAN A FUNCTION OF THE PATTERN OF COLD EXPOSURE. THIS IS IN CLOSE AGREEMENT WITH THE WORK OF LAWRENCE (28, 29), WENT (59) AND CAVERT (8). TEUBNER AND WITTWER (52) HAVE PRESENTED EVIDENCE THAT IT IS THE ROOT TEMPERATURE THAT ACTUALLY CONTROLS FLOWER NUMBER REGARDLESS OF THE TEMPERATURE TO WHICH THE TOPS OF THE PLANT ARE EXPOSED.

KRAMER AND WENT (25) FOUND A CLOSE CORRELATION BETWEEN THE AUXIN CONTENT OF TOMATO STEM TIPS AND EXPOSURE OF PLANTS TO LOW (8°C) AS COMPARED WITH HIGH TEMPERATURE (22°C). THEIR RESULTS, WHICH WERE BASED ON DIFFUSION COEFFICIENTS, FURTHER INDICATED THAT THE AUXIN FOUND IN TOMATO STEM TIPS IS 3-INDOLE ACETIC ACID WITH SOME OTHER HIGH MOLECULAR WEIGHT COMPOUNDS OCCURRING IN LOW CONCENTRATIONS. SUBSEQUENT STUDIES IN THIS AREA HAVE FAILED TO CONFIRM THESE FINDINGS ALTHOUGH OTHER UNIDENTIFIED AUXINS HAVE BEEN FOUND TO OCCUR IN TOMATO (5, 7, 14, 56). HOWEVER, ROW ET AL. (45) HAVE RECENTLY REPORTED THE ISOLATION OF 3-INDOLEACETYLASPARTATE FROM TOMATO SEEDLINGS.

STUDIES BY ANDREAE ET AL. (1) AND ROW ET AL. (45) INDICATE THAT PRODUCTION OF 3-INDOLE-ACETYL D, L ASPARTIC ACID MAY BE A DETOXIFICATION MECHANISM IN PLANTS FOR EXCESS AMOUNTS OF EXOGENOUSLY APPLIED IAA¹. THE LATTER GROUP HAVE ALSO HYPOTHESIZED THAT THIS CONJUGATION OF IAA AND ASPARTIC ACID MAY FUNCTION IN THE FLOWERING MECHANISM OF CERTAIN PHOTORESPONSIVE PLANTS.

THE PHYSIOLOGY OF FLOWERING IN LONG DAY AND SHORT DAY PLANTS HAS BEEN REVIEWED BY LANG (26,27). HOWEVER, RELATIVELY LITTLE RESEARCH HAS BEEN CONDUCTED ON THE FLOWERING OF SO-CALLED DAY NEUTRAL AND INTERMEDIATE DAY-LENGTH PLANTS (THOSE WHICH REQUIRE A PHOTOPERIOD OF BETWEEN 12 AND 16 HOURS). THE TOMATO FALLS INTO THE LATTER CLASSIFICATION (35, 42, 59).

LIVERMAN IN A RECENT REVIEW SUMMARIZED THE ROLE OF AUXINS IN THE FLOWERING OF LONG AND SHORT DAY PLANTS: "THE AUXIN LEVEL FALLS DURING THE DARK PERIOD OF LONG DAY PLANTS AND SHORT DAY PLANTS. IN SHORT DAY PLANTS, THE DARK PERIOD ALLOWS THE CONCENTRATION OF AUXIN TO FALL TO A LEVEL WHERE FLORIGEN SYNTHESIS MAY OCCUR. FOR LONG DAY PLANTS, ON THE OTHER HAND, THE DATA SUGGESTS THAT THEIR AUXIN LEVEL MAY BE TOO LOW FOR FLOWERING IN SHORT DAY CONDITIONS AND THAT BY ARTIFICIAL APPLICATION OF AUXIN, FLOWERING MAY ENSUE" (34). THIS IS BASED IN PART ON THE FACT THAT EXOGENOUS AUXIN APPLICATIONS HAVE AN INHIBITORY EFFECT ON FLOWERING IN

¹/ THE FOLLOWING ABBREVIATIONS WILL BE USED: TIBA, 2,3,5 TRIIODOBENZOIC ACID; IAA, INDOLE-3-ACETIC ACID; N-M-T, N-META-TOLYLPHTHALAMIC ACID.

SHORT DAY PLANTS. HOWEVER, LANG (27) INDICATES THAT A CLEAR RELATIONSHIP BETWEEN AUXIN LEVELS AND FLOWERING HAS NOT BEEN COMPLETELY SUBSTANTIATED. THE USE OF ANTI-AUXINS OR AUXIN ANTAGONISTS TO INCREASE FLORAL PROMOTION WOULD SEEM TO INDICATE A RELATIONSHIP BETWEEN AUXIN LEVELS AND THE FLOWERING MECHANISM (26, 27).

LANG (27) SPECULATES THAT LOW TEMPERATURE, BY RETARDING GROWTH, LEADS TO AN ACCUMULATION OF GREATER AUXIN LEVELS IN THE GROWING POINT WHICH FAVORS BOTH FLORAL INITIATION AND SUBSEQUENT VEGETATIVE GROWTH. THE INHIBITORY EFFECTS OF AUXIN APPLICATIONS ON FLOWER FORMATION IS EXPLAINED BY ASSUMING THAT THE APPLIED AUXIN STIMULATES VEGETATIVE GROWTH WHICH IN TURN INHIBITS FLOWER FORMATION.

NUMEROUS PAPERS HAVE REPORTED ON THE USE OF AUXINS TO INCREASE THE FLOWERING OF PLANTS. IN GENERAL IT SHOULD PROBABLY BE MENTIONED, HOWEVER, THAT IN MOST OF THE WORK REPORTED IN WHICH INCREASES IN FLOWER NUMBERS WERE OBSERVED, EXTREMELY LOW CONCENTRATIONS OF AUXINS WERE USED (USUALLY LESS THAN 100 PPM) AND THAT THE TIME OF APPLICATION WAS OF CRITICAL IMPORTANCE. LEOPOLD AND GUERNSEY (32) HAVE DEMONSTRATED THAT VERY LOW CONCENTRATIONS OF NAPHTHALENE ACETIC ACID (LESS THAN 1 PPM) APPLIED IN CONJUNCTION WITH LOW TEMPERATURES (3°C) RESULTED IN LARGE INCREASES IN FLOWER NUMBERS IN BARLEY. SIMILAR RESULTS WERE ALSO OBTAINED BY LEOPOLD AND THIMANN (33) USING IAA.

SEVERAL WORKERS (49, 50) HAVE SHOWN THAT APPLICATION OF LOW CONCENTRATIONS OF AUXINS RESULT IN EARLIER FLOWERING

AS WELL AS INCREASED NUMBERS OF FLOWER BUDS IN TOMATOES. HOWEVER, THIS MAY NOT BE EVIDENT IN THE NUMBER OF FLOWERS OF A MATURE INFLORESCENCE SINCE DEVELOPMENT OF THE IMMATURE BUDS IS APPARENTLY SUPPRESSED BY THESE AUXIN LEVELS (51). EFFECTS ON FLOWER FORMATION IN THE TOMATO HAVE ALSO BEEN OBTAINED WITH OTHER COMPOUNDS WHICH ARE GENERALLY NOT ACCEPTED AS TYPICAL AUXINS. BOTH 2,3,5 TRIIODOBENZOIC ACID (15, 65, 66) AND (2-NAPHTHOXY) PHENYLACETIC ACID (40) AS WELL AS VARIOUS N-ARYLPHTHALAMIC ACIDS HAVE BEEN FOUND TO INCREASE THE NUMBER OF FLOWERS WHICH DEVELOP IN A TOMATO INFLORESCENCE (51). THIS LATTER GROUP OF COMPOUNDS AND ESPECIALLY THE N-M-TOLYLPHTHALAMIC ACID DERIVATIVE HAVE BEEN SUCCESSFULLY USED WITH GREENHOUSE GROWN TOMATOES TO INCREASE FRUIT YIELDS AS A RESULT OF THE INCREASED FLOWER NUMBERS (62).

ZIMMERMAN AND HITCHCOCK (65) FIRST REPORTED THE EFFECTIVENESS OF TIBA IN FLOWER PROMOTION IN THE TOMATO. THEY FOUND THAT THE TREATMENT NOT ONLY INCREASED FLOWER NUMBERS, BUT ALSO RESULTED IN EARLIER FLOWERING AND AT HIGH CONCENTRATIONS COMPLETE "TERMINATION" OF THE PLANT IN A LARGE FLOWER CLUSTER. THEY SUGGESTED THAT TIBA REDUCED AUXIN LEVELS IN THE PLANT AND ITS EFFECT ON FLOWERING WAS INTERPRETED AS MEANING THAT THE NORMAL LEVEL OF ENDOGENOUS AUXIN IN THE TOMATO PLANT WAS INHIBITORY TO FLOWER FORMATION.

DEZEEUW (12), HOWEVER, FOUND THAT TIBA PROMOTES FLOWERING IN THE TOMATO ONLY IF YOUNG, GROWING LEAVES ARE

PRESENT ON THE PLANT. IF THESE YOUNG LEAVES WERE REMOVED, TIBA ACTUALLY INHIBITED FLORAL INITIATION. IN CONTRAST, 1-NAPHTHALENE ACETIC ACID AT LOW CONCENTRATIONS PROMOTED FLOWERING IN THE ABSENCE OF YOUNG LEAVES, BUT INHIBITED FLOWERING WHEN LEAVES WERE PRESENT. DeZEEUW ASSUMED AN ANTAGONISM BETWEEN YOUNG LEAVES AND THE DEVELOPING INFLORESCENCE, POSSIBLY IN COMPETING FOR AVAILABLE AUXINS. TIBA WAS THOUGHT TO HASTEN THE MATURITY OF THE YOUNG LEAVES AND THEREBY DECREASE THE COMPETITIVE EFFECT OF THESE ORGANS. IF THESE INTERPRETATIONS ARE CORRECT, AUXINS WOULD APPEAR TO BE REQUIRED FOR FLOWER FORMATION.

THE N-ARYLPHTHALAMIC ACIDS HAVE BEEN EXTENSIVELY STUDIED BY TEUBNER AND WITTWER (50, 51, 53) IN RELATION TO THEIR FLOWER PROMOTING EFFECTS ON TOMATO PLANTS. SHEN (46) FOUND THAT DEVELOPMENT OF THE SYMPODIAL SHOOT WAS TEMPORARILY REDUCED OR RETARDED AFTER TREATMENT, OFFERING A POSSIBLE EXPLANATION FOR THE ACTIVITY OF THIS COMPOUND IN INCREASING FLOWERING IN CONNECTION WITH DeZEEUW'S HYPOTHESIS PREVIOUSLY DISCUSSED. DeZEEUW ALSO WORKING WITH N-M-T FOUND THAT IT HASTENED LEAF AGEING IN TREATED TOMATO PLANTS AS DID TIBA, BUT WITH LESS INHIBITION OF LEAF GROWTH. HE FOUND WHEN N-M-T WAS APPLIED AS A LANOLIN PASTE (1%) TO THE APEX OF TOMATO PLANTS, AN ACTUAL REDUCTION IN FLOWER NUMBER RESULTED IF YOUNG LEAVES WERE REMOVED AND A SLIGHT INCREASE RESULTED WHEN THE YOUNG LEAVES WERE LEFT ON THE PLANT.

STRUCTURE ACTIVITY RELATIONSHIPS OF AUXINS

CONSIDERABLE INTEREST HAS BEEN SHOWN IN THE GENERAL AREA OF MOLECULAR STRUCTURE AND BIOLOGICAL ACTIVITY RELATIONSHIP IN AUXINS AND "AUXIN-LIKE" COMPOUNDS. IT SEEMS THAT AT PRESENT THERE IS NO FIRM BASIS FOR THE FORMULATION OF EXACT RULES FOR THE STRUCTURE-ACTIVITY RELATIONSHIP OF AUXINS DUE TO THE COMPLEXITY AND DIVERSITY OF THE VARIOUS GROUPS OF AUXINS AND THE POSSIBLE VARIATION IN MECHANISM BETWEEN THE DIFFERENT GROUPS. HOWEVER, ATTEMPTS HAVE BEEN MADE TO GENERALIZE STRUCTURAL REQUIREMENTS OF AUXINS AND HAVE PROBABLY HAD A PROFOUND EFFECT ON SUBSEQUENT STUDIES IN THIS AREA.

EARLY INVESTIGATIONS REVEALED SEVERAL STRUCTURAL FEATURES WERE REQUIRED FOR AUXIN ACTIVITY. THE FIVE REQUIREMENTS CONSIDERED ESSENTIAL FOR AUXIN ACTIVITY BY WENT AND THIMANN IN 1937 (60) WERE:

1. A RING SYSTEM AS THE NUCLEUS
2. AT LEAST ONE DOUBLE BOND IN THIS RING
3. A SIDE CHAIN CONTAINING A CARBOXYL GROUP (OR A GROUP EASILY CONVERTIBLE TO A CARBOXYL GROUP)
4. THERE MUST BE AT LEAST ONE CARBON ATOM BETWEEN THE CARBOXYL GROUP AND THE RING
5. A PARTICULAR SPACIAL RELATIONSHIP BETWEEN THE RING AND THE CARBOXYL GROUP.

VELDSTRA (56) SUBSEQUENTLY CONDENSED THESE REQUIREMENTS INTO THE FOLLOWING:

1. A BASAL RING SYSTEM WITH HIGH SURFACE ACTIVITY
2. A POLAR GROUP OF ACID CHARACTER IN A VERY DEFINITE SPATIAL POSITION WITH RESPECT TO THIS RING SYSTEM.

VELDSTRA ALSO POSTULATED THAT A CERTAIN LIPOPHILIC-HYDROPHILIC BALANCE BETWEEN THE TWO PARTS OF THE MOLECULE WAS ESSENTIAL.

A RECENT THEORY PROPOSED BY WAIN (58) CONSIDERS AUXINS OF THE ARYLALKYL- AND ARYLOXYALKYL CARBOXYLIC ACID TYPES BUT NOT THE ARYLCARBOXYLIC ACIDS SUCH AS THE BENZOIC ACID SERIES. HE CONCLUDES THAT AN ACTIVE MOLECULE OF THESE SERIES MUST INCLUDE 1) A FLAT RING SYSTEM, 2) A HYDROGEN ATOM ON THE ALPHA CARBON OF THE CARBOXYLIC ACID SIDE CHAIN, 3) A SPECIAL CONFIGURATION OF THE SIDE CHAIN WITH RESPECT TO THE RING, AND FINALLY 4) FREE ROTATION OF THE SIDE CHAIN ABOUT THE BOND JOINING IT TO THE RING. THESE REQUIREMENTS WERE INTERPRETED AS INDICATING A THREE POINT ATTACHMENT AT THE SITE OF ACTIVITY.

JONSSON (23, 24) MADE A SIMILAR INTERPRETATION OF THE STRUCTURAL REQUIREMENTS ALTHOUGH MUCH MORE DETAILED IN DESIGN. HE RECENTLY MODIFIED THESE EARLIER REQUIREMENTS TO THE FOLLOWING:

1. AN UNSATURATED RING NUCLEUS OR OTHER ATOMIC ARRANGEMENT FORMING A FLAT AREA OF REASONABLE EXTENSION.

2. A SIDE CHAIN CARRYING A CARBOXYL GROUP OR OTHER ACIDIC GROUP WHICH MUST NOT BE SITUATED AT A QUATERNARY CARBON ATOM OR OTHERWISE GREATLY HINDERED STERICALLY.
3. THE SIDE CHAIN MUST BE ABLE TO ASSUME A SPACIAL ORIENTATION IN WHICH THE ATOMS JOINING THE RING AND THE CARBOXYL GROUP, INCLUDING THE CARBON ATOM OF THE LATTER, ARE SITUATED ALMOST IN THE PLANE OF THE RING FORMING A PSEUDO-RING WITH THE CARBOXYL GROUP CLOSE TO THE ORIGINAL RING AND NEAR THE CENTER OF THE EXTENDED RING SYSTEM THUS FORMED.
4. AT LEAST ONE SIDE OF THE PLANE OF THE EXTENDED RING SYSTEM MUST BE FREE OF ATOMS OTHER THAN HYDROGEN AND ONE OF THE CARBOXYLIC OXYGEN ATOMS PROJECTING OUT FROM IT.
5. THE EXTENDED RING SYSTEM MUST HAVE A HIGH INTERFACIAL ACTIVITY.
6. AN OPTICAL CONFIGURATION CORRESPONDING TO THE D-SERIES OF THE AMINO ACIDS.

THESE REQUIREMENTS WERE THOUGHT BY JONSSON TO COVER ALL GROUPS OF AUXINS INCLUDING COMPOUNDS NOT POSSESSING A RING NUCLEUS. THESE REQUIREMENTS ARE QUITE SIMILAR TO THOSE DESCRIBED BY WAIN EXCEPT THAT THE HYDROGEN ATOM ON THE ALPHA CARBON IS NOT SPECIFICALLY REQUIRED BUT RATHER

THAT THERE MUST NOT BE A GROUP PRESENT AT THIS POSITION WHICH COULD CAUSE STERIC HINDRANCE AT THE RECEPTOR SITE.

A SOMEWHAT DIFFERENT APPROACH TO THIS PROBLEM HAS BEEN POSTULATED BY MUIR AND HANSCH (38) WHO ENVISION A CHEMICAL REACTION OCCURRING BETWEEN AN UNSUBSTITUTED CARBON ATOM IN THE AUXIN RING ORTHO TO THE SIDE CHAIN AND A SULF-HYDRYL GROUP OF THE PROTEIN AT THE SITE OF ACTION. A SECOND REACTION BETWEEN THE CARBOXYL GROUP OF THE AUXIN AND AN AMINO GROUP OF THE PROTEIN WAS ALSO POSTULATED.

THIS FREE ORTHO POSITION HAS SINCE BEEN MODIFIED TO BE A REQUIREMENT ONLY FOR THE PHENOXYACETIC ACIDS. IN THE BENZOIC ACID SERIES WHERE THE 2, 6 DISUBSTITUTED FORMS SHOW ACTIVITY, THEY ASSUME THAT THE ACTIVATING INFLUENCES OF THE CARBOXYL GROUP FACILITATED NUCLEOPHILIC REPLACEMENT OF ONE CHLORINE ATOM. THEY FOUND INCREASED CHLORIDE ION CONTENT OF TISSUE TREATED WITH 2,6 DI-CHLOROBENZOIC ACID AND THIS IS CITED AS SUPPORTING EVIDENCE FOR THIS REACTION (38). THE FEASIBILITY OF THIS IS QUESTIONABLE, HOWEVER, SINCE INCREASED CHLORIDE CONTENT WAS FOUND FROM BOTH ACTIVE AND INACTIVE FORMS, SUCH AS THE 2,4-DI-CHLOROBENZOIC ACID DERIVATIVE (38).

SEVERAL WORKERS (30, 43) HAVE POSTULATED VARIOUS METABOLICALLY CONTROLLED CHANGES IN THE STRUCTURE OF THE AUXIN MOLECULE, BUT AS JONSSON (24) POINTS OUT, IT IS DIFFICULT TO FIND REACTIONS WHICH ARE LIKELY TO OCCUR IN ALL THE DIVERSE TYPES OF COMPOUNDS WHICH ARE KNOWN TO POSSESS AUXIN ACTIVITY.

BIOLOGICAL ACTIVITY OF THE N-ARYLPHTHALAMIC ACIDS

THE "FRUIT-SETTING" ACTIVITY OF A NUMBER OF DERIVATIVES OF THE N-ARYLPHTHALAMIC ACIDS WAS FIRST REPORTED IN 1949 BY HOFFMAN AND SMITH (20). THE SUBSEQUENT DISCOVERY BY TEUBNER AND WITTWER (50) OF THE FLOWER PROMOTING EFFECT OF THE N-M-T DERIVATIVE WHICH RESULTED IN INCREASED YIELDS OF GREENHOUSE GROWN TOMATOES STIMULATED FURTHER INTEREST IN THIS GROUP OF COMPOUNDS.

A NUMBER OF CHLORO- AND METHYL SUBSTITUTED N-PHENYL-PHTHALAMIC ACIDS WERE FOUND TO POSSESS FLOWER FORMING ACTIVITY AND THE STRUCTURAL REQUIREMENTS OF THIS GROUP OF COMPOUNDS FOR ACTIVITY IN FLOWERING DIFFERED SOMEWHAT FROM OTHER "AUXIN-LIKE" COMPOUNDS (52). THEY SUBSEQUENTLY FOUND THAT MODIFICATION IN MOLECULAR STRUCTURE RESULTED IN SIMILAR RESPONSES IN TOMATO FLOWERING, PARTHENO-CARPIC DEVELOPMENT OF TOMATO OVARIES, AND THE AVENA COLEOPTILE STRAIGHT GROWTH BIOASSAY (54). THEY CONCLUDED THAT BOTH ORTHO AND META SUBSTITUTION OF CHLORO- OR METHYL GROUPS ENHANCED THE ACTIVITY OF N-PHENYLPHTHALAMIC ACID AND FOUND THE 2,3 AND 3,5 DICHLORO- AND THE 2,3,5 TRICHLOROPHENYLPHTHALAMIC ACIDS WERE THE MOST ACTIVE TESTED. IN CONTRAST, PARA AND DI-ORTHO SUBSTITUENTS EITHER REDUCED OR HAD NO EFFECT ON BIOLOGICAL ACTIVITY (54). THEY CONCLUDED THAT MODIFICATION OF STRUCTURE THROUGH VARIOUS SUBSTITUTIONS OF METHYL- AND CHLORO GROUPS ON THE PHENYL RING HAD THE SAME EFFECTS ON AUXIN ACTIVITY AS ON ACTIVITY IN TOMATO FLOWER FORMATION. ON THE BASIS OF

THESE OBSERVATIONS, TEUBNER ET AL. (54) HAVE HYPOTHESIZED THAT THE N-ARYLPHTHALAMIC ACIDS ACT AS AUXINS IN THEIR EFFECTS ON TOMATO FLOWERING.

GULLSTROM ET AL. (17) CONDUCTING RESIDUE STUDIES ON N-M-T FOUND THAT RAPID HYDROLYSIS OF THE MOLECULE OCCURRED RESULTING IN THE FORMATION OF THE FREE PHTHALIC ACID AND M-TOLUIDINE. THEY FOUND THAT THE RATE OF HYDROLYSIS RAPIDLY INCREASED WITH INCREASING TEMPERATURE AND LOWER PH. TEUBNER AND WITTWER (50) REPORTED THAT SOLUTIONS OF N-M-T STORED FOR ONE TO TWO DAYS HAD NO EFFECT ON TOMATO FLOWER FORMATION. SHEN (46) ALSO STUDIED THE EFFECTS OF AGEING ON ACTIVITY OF N-M-T AND FOUND THAT THE CHEMICAL WAS INEFFECTIVE IN INCREASING FLOWERING IN TOMATO AFTER FOUR DAYS. HER STUDIES WERE INCONCLUSIVE AS TO WHETHER THE INACTIVATION OF THE SOLUTION WAS DUE MERELY TO HYDROLYSIS OR WHETHER POSSIBLE INTRAMOLECULAR CHANGES RESULTED IN AN INACTIVE FORM.

MECHANICAL HARVESTING OF TOMATOES

THE RECENT DEVELOPMENT OF MECHANICAL HARVESTING EQUIPMENT FOR FIELD GROWN TOMATOES HAS STIMULATED CONSIDERABLE INTEREST IN THIS AREA. RIES AND STOUT (44) HAVE REPORTED CONSIDERABLE PROGRESS IN THE DEVELOPMENT OF THIS HARVESTER, BUT ALSO POINT OUT SEVERAL PROBLEMS WHICH MUST BE RESOLVED BEFORE PRACTICAL USE OF THESE MACHINES BECOMES A REALITY. FIRST AND FOREMOST AMONG THESE PROBLEMS IS THAT THE MACHINE BEING DEVELOPED CUTS OFF THE PLANTS AT GROUND

LEVEL AND SUBSEQUENTLY SHAKES THE FRUIT LOOSE FROM THE VINES. THIS ALLOWS ONLY ONE HARVEST PER PLANT AS COMPARED WITH THE PRESENT SYSTEM OF SEVERAL HAND HARVESTS. THUS THE "CONCENTRATION" OF YIELDS INTO A LARGE SINGLE HARVEST IS NECESSARY IN ORDER TO OBTAIN YIELDS OF SUFFICIENT MAGNITUDE WITH THE MECHANICAL HARVESTER.

ONE APPROACH TO THIS PROBLEM IS THROUGH THE DEVELOPMENT OF CULTIVARS WHICH NATURALLY DEVELOP CONCENTRATED YIELDS. CONSIDERABLE PROGRESS HAS BEEN MADE THROUGH THE DEVELOPMENT OF EPOCH AND CLOSELY RELATED STRAINS. THESE SEMI-DWARF TYPE PLANTS CAN BE PLANTED AT VERY HIGH PLANT POPULATIONS WHICH RESULT IN HIGH YIELDS PER ACRE FOR A SINGLE HARVEST ALTHOUGH YIELDS PER PLANT ARE QUITE LOW.

A SECOND APPROACH TO THIS PROBLEM OF CONCENTRATING YIELDS IS THROUGH THE USE OF CHEMICALS SUCH AS N-M-T WHICH INDUCE INCREASED FLOWER NUMBERS PER CLUSTER AND THEREBY INCREASE THE NUMBER OF FRUIT UNIFORMLY RIPENING ON A PLANT. THE RESULTS OBTAINED FROM THE USE OF THIS CHEMICAL WITH GREENHOUSE GROWN TOMATOES INDICATE THAT THESE COMPOUNDS CAN BE USED ON A PRACTICAL BASIS IN ORDER TO INCREASE YIELDS BY INCREASING THE NUMBER OF FLOWERS PER INFLORESCENCE.

MATERIALS AND METHODS

TOMATO FLOWERING STUDIES

IN ALL GREENHOUSE STUDIES, UNLESS OTHERWISE INDICATED, THE CULTIVAR MICHIGAN-OHIO HYBRID WAS USED THROUGHOUT THESE INVESTIGATIONS. IT IS IMPORTANT TO NOTE THAT THIS CULTIVAR, DESPITE ITS HORTICULTURAL NAME, MAY NOT REPRESENT A TRUE HYBRID WHICH IN BOTANICAL NOMENCLATURE IS RESTRICTED TO PRODUCTS OF INTERSPECIFIC CROSSES. ON THE OTHER HAND, HIGH RESISTANCE TO FUSARIUM WILT SUGGESTS THAT *L. PIMPINELLIFOLIUM* MAY HAVE BEEN INVOLVED IN ITS ANCESTRY. SINCE THE ANCESTRY OF THE TWO IMMEDIATE PARENTS, MICHIGAN STATE FORCING AND OHIO WR 3, IS OBSCURE, IT IS LOGICAL TO REFER TO THIS CULTIVAR AS A HALF-BREED RESULTING FROM A CROSS WITHIN THE SPECIES *L. ESCULENTUM*. SEEDS WERE SOWN IN STERILIZED METAL FLATS CONTAINING PRESOAKED VERMICULITE. THE SEEDS WERE USUALLY GERMINATED AT 75 TO 80°F AND THEN TRANSFERRED TO A GREENHOUSE WITH A TEMPERATURE OF 75°F DURING CLEAR DAYS AND 65°F DURING CLOUDY DAYS. THE NIGHT TEMPERATURE WAS MAINTAINED AT 65°F THROUGHOUT THE DEVELOPMENT OF THE PLANTS. THE SEEDLINGS WERE TRANSPLANTED TO STERILIZED 4-INCH CLAY POTS OF SOIL ABOUT THE TIME OF COTYLEDON EXPANSION AND THEN TO 8-INCH CLAY POTS OF SOIL WHEN THE PLANTS WERE APPROXIMATELY 12 INCHES HIGH. THE SOIL USED THROUGHOUT THE EXPERIMENT WAS A 2:2:1 STEAM STERILIZED MIXTURE OF SILTY CLAY LOAM SOIL, COMPOST AND SAND RESPECTIVELY. THE SEEDLINGS WERE FED