NITROGEN METABOLISM IN POLLEN

by

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INTRODUCTION

Pollen germination and tube growth are important manifestations of living activities of the pollen grain. Growth in other plant cells is associated with a net synthesis of protein molecules, and one would expect this to be true in pollen also. When the pollen grain germinates and the tube grows, the increase in volume, as compared with the original volume of the pollen grain, is enormous. And yet, such an increase is not associated with a commensurate increase in the amount of cytoplasm. This is because of the formation of a large vacuole, which periodically becomes separated from the mass of the cytoplasm by plugs of callose. A vacuole is formed between the callose plug and the cytoplasm followed by the formation of another callose plug. This pattern of alternate vacuole and plug formation continues to follow the cytoplasm as it moves with the growing pollen tube tip, until, before the tube is very long, the cytoplasm occupies only a small volume near the tip and the remainder is vacuole partitioned off by callose plugs.

The control of metabolism in living cells is known to rest with DNA, present in the chromosomes of the nucleus. The RNA synthesized in the nucleus is responsible for protein synthesis. The protein molecule, once formed, does not remain intact for the life of the cell. On the contrary the life of each individual molecule is definitely limited and protein molecules are continually being synthesized and broken down. Thus one would not expect the protein content to remain constant during tube development, unless the rates of synthesis and breakdown were identical. If no synthesis were occurring, then the protein content of the tube would decrease with tube development. One exception to this statement would be if the protein synthesized by the pollen should leave the pollen and move into the external environment.

Since there does not appear to be any great increase in the amount of cytoplasm during pollen tube development, but since protein synthesis should occur during growth this presents an inconsistent situation that should be clarified. Attempts to demonstrate the net synthesis of protein during tube development have left us with conflicting reports. And yet, this is such an important living activity that if protein synthesis were not occurring, the pollen tube would certainly be unique among living cells.

Since radioactive leucine was made available to us, we investigated this phenomenon to answer the question of whether or not protein synthesis occurred during pollen tube development. The results of this study indicate that protein synthesis did occur during pollen tube development, and that some of this synthetic activity did result in a net increase in protein.

SURVEY OF LITERATURE

Nitrogen Requirement for In Vitro Germination and Tube Growth of Pollen

Since Von Mohl in 1834 initiated in vitro germination tests for pollen, investigators have studied the effects of many substances, both organic and inorganic, on pollen germination and tube growth. Johri and Vasil (1961) have reviewed the literature pertaining to the effects of some of these substances on pollen germination and tube growth. While pollen of some plant species is able to grow without an exogenous supply of nutrients, others require specific chemical substances. The need of boron and sucrose for in vitro growth of pollen grains is well established.

The effect of various nitrogen sources on a variety of organisms and plant tissues have been studied. Harvey (1953) reported that synthesis of organic nitrogen in the marine diatom, Nitzchia closterium, proceeded rapidly from ammonium, less rapidly from nitrate and very slowly from nitrite in the dark. In a study of the effect of a variation in the nitrogen sources on induced enzyme formation, Franke et al. (1965) concluded that the best sources of nitrogen were nitrates, different amino acids, uric acid and urea. The pH was considered critical in this assessment. Grover and Sidhu (1965) found that the fungus Pythium aphanidermatum assimilated amino and nitrate nitrogen better than ammonium nitrogen while nitrite was not utilized.

Reports of investigations on the effect of different nitrogen sources on pollen germination and growth are also available. Brink (1924) confirmed the observations of Lidforss (1896) that calcium nitrate and potassium nitrate were toxic to pollen grains even at low concentrations.

Certain amino acids, on the other hand, were found to have stimulating effects on the growth of pollen tubes. Tulecke (1960) studied arginine-

requiring strains obtained from Ginkgo pollen tissue. These strains grew best with arginine as the source of nitrogen but, in decreasing order, NH_3, lysine, ornithine or urea could be used. While proline promoted growth, hydroxyproline was inhibitory. Sawada (1958) found that aspartic-, glutamic-, and alanine- transaminases stimulated in vitro germination of Zea mays pollen. Amino acids, however, were toxic in higher concentrations, with the optimum concentrations varying with different amino acids (Lensu 1963). It is known in certain organisms that amino acids cannot be utilized for protein synthesis unless supplied as a combination of a number of amino acids. The exogenously supplied single amino acids may be toxic because of the inability of the pollen grain to utilize them in protein synthesis, and, as a consequence of deamination, NH_3 may accumulate in high concentrations in the pollen grain. Exogenous proline, at optimum concentration, stimulated germination and growth of pollen tubes of Antirrhinum majus, Salix caprea, Petunia hybrida and Tradescantia virginiana (Britikov et al. 1966).

These studies indicate that different responses are obtained by varying the nitrogen sources and that the same group of compounds may either be toxic or may stimulate germination and growth of pollen tubes.

Proteins and Enzymes of Pollen

The protein content of pollen has been investigated extensively and efforts to characterize it have been made mainly from the point of view of recognizing the allergenic principle in pollen. Ragweed (Ambrosia sp.) pollen has been recognized as a causative agent of common seasonal hayfever. Most investigators of allergenic pollen employ pollen of this genus. The allergenic activity is known to be associated with either the protein or peptide fraction of pollen.

As early as 1803, it was observed by Fourcroy and Vauquelin and others, that pollen is rich in protein. Planta (1884, 1886) reported 16.6 percent protein in Pinus sylvestris and 30.1 percent in Corylus avellana. Heyl's (1917) analysis of ragweed pollen showed 24.4 percent proteins. The albumin and globulin fractions were small but "proteoses" were predominant. The chief protein was glutelin. Hydrolysis of albumin, glutelin and proteoses yielded various amino acids (Heyl 1920). Czapek (1920) observed that the protein content of pollen ranged between 1 - 30 percent.

Sehon and Richter (1959) separated a fraction from short ragweed pollen which was shown to contain a pigment and polypeptides. This fraction upon hydrolysis was composed of 8 amino acids: arginine, lysine, glutamic acid, glycine, alanine, hydroxyproline, valine and norleucine.

Nasatir, et al. (1960) observed that soluble proteins from Lilium anthers contained four electrophoretic components, which showed quantitative changes during the development of the anther. Callaghan and Goldfarb (1962) isolated a protein containing sulfhydryl groups from dwarf ragweed pollen.

Protein content in other genera have been reported. Sweet corn has 26.88 percent protein when expressed on moisture-, ash-, and lipid-free basis (Sarkar et al. 1949). Although the protein content of Zea mays, Alnus glutinosa and Pinus montana showed quantitative differences, no great differences in amino acid composition was observed (Nielsen et al. 1955). Chemical analysis of pollen from Pinus canariensis gave 7.6 percent protein, 5.3 percent free amino acid and 1.95 percent nitrogen. Lubliner-Mianowska (1956) reported 20-30 percent crude protein in more than half of the 17 plant species he studied. In an investigation of protein and vitamin E content as related to fertilization, Shpakova (1961) observed a positive correlation Histochemical investigation of pollen of some angiosperms revealed that

larger amounts of protein and amino acids were found in non-germinating than in germinating pollen. (Poddubnaya - Arnoldi et al. 1961)

Stanley and Linskens (1965) showed that diffusates of petunia pollen contained a Lowry - reacting material shortly after being placed on the germinating medium. Judging by the absorption peak at 285μ they suggested that this may be protein but did not rule out the possibility of it being a nucleotide or a flavonoid. More recently, however, Lewis, et al. (1967) noticed protein diffusion from single, intact pollen grains of Oenothera organensis within 2·5 minutes. In one hour, as much as 40 percent of the total protein diffused out.

Linskens (1966) studied the variation in patterns of proteins and enzymes during the development of pollen. Sixteen stages of development were classified and it was noticed that both protein and enzyme patterns had a phase specificity.

The ability of the pollen grain to metabolize nutrients supplied exogenously and the fact that the pollen tube descends down the style by the digestion of the stylar tissue led to the belief that pollen grains secrete enzymes. Pollen enzymes have been studied mainly in quest of an answer to the phenomenon of incompatibility in plants. Histochemical research points to a correlation that may exist between the pollen enzymes and incompatibility.

Chauvshina and Melhikov (1964) studied the first generation hybrids of intergeneric crosses of representatives of Rosaceae and Grossulariaceae with a view to determining the causes of male sterility. Cytochemical determination of activity of certain oxidizing enzymes and the contents of physiologically active and reserve substances showed that in the sterile pollen of hybrids there was no active cytochrome oxidase, while in the pollen of the initially fertile forms, activity of this enzyme was very

high. On the other hand, the activity of the peroxidase in the fertile pollen was lower than in the sterile pollen.

Makinen and Brewbaker (1967) observed a diffusion of esterases, amino-peptidases, catalases, amylases and acid phosphatases out of intact and non-germinated pollen grains of Oenothera organensis.

Yet another area, to which a study of pollen enzymes has been applied, is evolution. In an analysis of data given by other workers in the field, Tsinger (1961) has drawn a correlation between the phylogenetic tree of angiosperms and the activity levels of respiratory enzymes. In lower and middle portions of the tree, when passing from lower towards higher forms of plants, the total activity of terminal oxidative enzymes increases and can be correlated with the position of the order in the system. However with higher orders of angiosperms, a sharp decrease of respiratory activities occurs when passing from lower towards higher forms of plants, representing a degeneration of pollen with progress in evolution. These reports do show that proteins are abundant in pollen, as are enzyme systems, and that proteins can move out of the pollen into the environment.

The physiology of sexual incompatibility is not fully understood and most of the explanations are only speculative. East (1929) propounded the immunity theory, suggesting an antigen-antibody reaction between the pollen and the pistil. Preformed substances in the style were thought to react with antigens from the pollen and the reaction products were believed to cause an inhibition of the pollen tubes.

The amazingly high content of proline in the pollen of a number of plants opened up a new area of investigation, namely, the role of amino acids in the reproductive system of plants. A comparison of the fertile and sterile genetical lines indicated a greater accumulation of free alanine in

normally developed fertile pollen than in sterile pollen (Khoo and Stinson, 1957). On the basis of tracer studies, Tupy (1961) concluded that one of the reasons for the arrest of growth of pollen tubes in incompatible crosses is a relative deficiency of γ-aminobutyric acid and alanine. Palilova and Bonsman (1964) showed by a cytochemical study of male sterility in corn, an increasing accumulation of free amino acids during microsporogenesis in sterile plants. Fertile plants were characterized by an absence of such free amino acids, indicating an interference with "protein synthesis".

Makinen and Brewbaker (1967) suggested that the early growth of pollen tubes may be influenced by the metabolic products of pistillate tissues caused by the diffused pollen enzymes. The inactivation of these enzymes by stigmatic or stylar components may lead to incompatibility reactions.

Simola (1967) studied the effects of some non-protein amino acids on the in vitro growth of pollen tubes in five species of the Vicieae. Canavanine and diaminobutyric acid inhibited pollen germination and pollen tube growth, implying that they may be antimetabolites of common amino acids. Non-Protein amino acids may thus form effective hybridization barriers. The ability to synthesize a new amino acid may, therefore, be of evolutionary significance in the isolation of new species and genera.

The studies indicate a close relationship between nitrogen metabolism in pollen and the phenomenon of incompatibility in plants. They seek to provide an explanation of incompatibility in terms of biochemical reactions.

POLLEN TUBE GROWTH AND PROTEIN SYNTHESIS

During the years 1824 to 1830, Amici (1824, 1830) made the fundamental discoveries that pollen grains germinated on the stigma and the pollen tubes grew into the style and ovary and finally entered the ovules.

Pollen grains of the average flowering plant germinate in a few minutes, with the tubes growing in situ at a rate ranging up to 1 cm/hr, averaging approximately 2 mm/hr for the species on which data are available. This amounts to cell elongation at least 250 times the pollen diameter in one hour. Biochemically, pollen appears to be well equipped, at least initially, for this rapid growth (Brewbaker and Emery 1961).

The pollen grain may be considered unique since it shows two forms of growth, the generation of two sperm nuclei from the generative nucleus, which may be regarded as cell proliferation, and the development of the tube which is a form of growth by cell enlargement. Protein synthesis is known to be required for cell division as well as for cell elongation (Nooden and Thimann 1963, 1965 and Key 1964).

The current concept of protein synthesis in plant cells centers on amino acid activation with ATP. The activated amino acid is then directed into place by interactions of messenger RNA, soluble RNA and ribosomal RNA. Messenger RNA, thought to be formed on DNA, is considered to be the template on which amino acids, attached to a series of sRNAs, are lined up to form proteins. Each strand of mRNA associates itself with five or more ribosomes to form polyribosomes, the site for protein synthesis.

Most of the amino acids needed for protein synthesis have been found in pollen and perhaps all are present but not detected. Seventeen free amino acids in the pollen of four species of grass have been examined by Bathurst (1954) using microbiological methods of assay. Sarkar et al. (1949) reported a total content of 12 amino acids estimated in the hydrolysis of the pollen of sweet corn, while Kanshina (1964) demonstrated eight amino acids in the pollen of cherry.

The metabolism of individual amino acids in pollen has been studied

by some workers. Britikov et al. (1965) found that radioactive proline was specifically incorporated into the collagen-like proteins of the protoplasm of growing pollen tubes. The proline that was not incorporated produced nitrogen which was utilized in the formation of new amino acids required for protein synthesis in the growing pollen tubes. Tulecke and Rutner (1965) report changes in the amino acid composition of the medium and cells of pollen tissue of Ginkgo during growth in a liquid medium containing arginine.

Both RNA and DNA have been found in pollen. Chang and Kivilaan (1962) reported 0.64 percent RNA and 0.04 percent DNA, on a dry weight basis, in pollen of Oenothera organensis.

Studies on the changes in nucleic acid content during pollen development are of significance in an investigation of protein synthesis. Linskens (1958) reported a decrease in DNA from 1.1 percent of the dry weight to 0.5 percent at the onset of prophase I in Lilium henryi pollen. During interphase I, DNA content returned to 1.1 percent. Rodkiewicz (1960), using Feulgen-photometry on pollen of Tradescantia bracteata, found that the DNA content in prophase and metaphase of the first division corresponded to that of a diploid cell. The amounts of DNA were similar in the vegetative and generative nuclei. The findings of Sato (1964) on the changes in the amount of DNA in pollen grains of T reflexa at various mitotic stages were in accord with the "DNA constancy" hypothesis. Turpin et al. (1965) found a direct proportionality between nucleic acid content in pollen of beet and the ploidy of the plant.

There are a number of reports of nucleic acid synthesis in the pollen grain during germination and pollen tube growth. Woodward (1958) pointed out that RNA synthesis occurs in pollen of Tradescantia during growth and

that both the nucleus and cytoplasm are participants in such synthesis. Young and Stanley (1963) obtained an incorporation of labelled nucleosides into both RNA and DNA of the generative and vegetative nuclei. Tano and Takahashi (1964) investigated nucleic acid synthesis in tobacco pollen during tube elongation using ^{32}P. The pollen incorporated orthophosphate ^{32}P, although the base composition of the newly synthesized RNA and that of the bulk RNA were at variance. A similar approach was adopted by Mascarenhas (1966), whose study indicated that uridine-^{3}H was incorporated into the vegetative and generative nuclei of Tradescantia paludosa.

Within the growing pollen grain, two nuclei are present. One is the tube nucleus and the other, the generative nucleus. Each is surrounded with cytoplasm to form the tube cell and the generative cell. These two cells, although initially derived from a common parent cell, are known to behave quite independently with regards to metabolic activity. The tube cell of Tradescantia pollen is known to contain large amounts of RNA and protein (Bryan 1951). Poddubnaya-Arnoldi et al. (1959) showed that in germinating angiosperm pollen, the tube cell and/or the tip of the germinating pollen grain secrete large amounts of enzymes and proteins. Evidence to suggest that the vegetative cell is functional rather than degenerate has been shown by Mascarenhas (1966).

Much headway has been made in the study of the mechanism of protein synthesis in growing pollen tubes. Tritiated nucleosides, radioisotopes and base analogues have been used for these studies. Protein synthesis in the pollen tubes per se was recently investigated by Poszar (1960), who reported that, during pollen tube growth, the total nitrogen content did not change and that the amount of pure protein underwent a rapid reduction. He, therefore, concluded that a de novo protein synthesis did not occur during pollen tube growth.

On the other hand, the capacity of the pollen tube to synthesize protein in vitro was established by Tupy (1963, 1964). That such a synthesis is not only due to turnover of pollen protein has been indicated by Tupy (1966).

We, therefore, have two different and conflicting views, namely: (1) that net protein synthesis does not occur during pollen tube growth, and (2) that net protein synthesis does occur at this time. For protein synthesis to occur, necessary s-RNA, m-RNA and ribosomal RNA must be present.

Woodward (1958) observed that in developing pollen, RNA content approximately paralleled amounts of protein formed. Data obtained by Mascarenhas (1966) and Larson (1965) indicate that both the vegetative nucleus and generative nucleus are active in synthesizing RNA. Young and Stanley (1963) had also reached the same conclusion. Although, whether it was RNA and DNA synthesis or turnover, was an open question.

The synthesis of RNA in the vegetative nucleus is enigmatic in view of the fact that DNA is synthesized only in the process of a doubling of the chromosomes (Moses and Taylor 1955) and that RNA synthesis in pollen is DNA dependent (Mascarenhas 1966). It may be suggested that some new DNA template would have to be synthesized during the growth of the pollen tubes. Findings of Tano and Takahashi (1964) may point in this direction. They found that the base composition of the newly synthesized RNA was entirely different from the bulk RNA in pollen and resembled that of DNA of this plant. They suggested that the RNA synthesized may be of the messenger type. It has been proposed by Mascarenhas (1966) and Tupy (1966) that the messenger RNA is of a stable long-lived type.

Inhibition of RNA synthesis by Actinomycin D (Mascarenhas 1966) by 2-thiouracil (Tupy 1966) and by other antimetabolities of nucleic acid bases

(Tupy 1966, Stanley and Linskens 1965) did not result in an inhibition of growth of pollen tubes as might have been expected. The DNA-dependent RNA synthesis may represent a small fraction of the total RNA synthesis of the growing pollen tube (Tupy 1966).

This view has led to the suggestion that protein synthesis, that is independent of DNA-dependent RNA synthesis, may occur in certain cells. In animal tissue, sea-urchin embryos (Gross, Malkin and Moyer 1964) have been known to retain the capacity to synthesize proteins in the absence of RNA synthesis among plants. Sieve tubes and germinating cotton seeds (Dure and Waters 1965) have been shown to possess such a mechanism. Tupy (1966) proposes such a mechanism in the pollen of pine.

Mascarenhas (1966) proposed that such RNA as is required for protein synthesis during germination and early growth of pollen tubes is synthesized before the pollen is released from the anthers. Woodward (1958) did in fact find a rapid accumulation of RNA and protein in the cytoplasm of the pollen grain 24 hours before anthesis.

The studies are indicative of the progress made towards resolving whether protein synthesis does occur during pollen tube growth and the time and mechanism of such synthesis.

Protein synthesis in growing cells

A study of the metabolism of nitrogen compounds during the elongation of cells was initiated by Frey-Wyssling and Blank (1940) who described a

[illegible] that the elongation of the coleoptile of maize. [illegible] coleoptile of maize, in which cell division plays a minor role, is accompanied by a 9·5 fold increase in protein content. Blank and Frey-Wyssling (1941) found that similar changes

occurred during cell enlargement in the hypanthium of Oenothera. Frey-Wyssling and Blank (1948) showed that elongating cells may increase their protein nitrogen greatly and rapidly by synthesis from soluble-nitrogen compounds even though the protein nitrogen relative to the fresh or dry weight is on the decline. Kopp (1948) calculated that, in the root, the protein content increased during elongation, and Brown and Broadbent (1950) demonstrated this condition experimentally in pea roots.

Steward et al. (1958) have indicated that when mature, non-dividing cells of the potato or carrot, which are rich in free amino acids such as asparagine and glutamine, are caused to grow by the use of growth factors found in coconut milk. The balance between soluble nitrogen and protein nitrogen changed in favor of protein.

Mathaei (1957) has domonstrated with petals that the protein content may keep pace with the increase in the dimension of the cells, exceed it or fall behind it, although the greatest increase in size occurred when the protein, either at the outset or throughout, showed an increase. There was no proportionality between growth and protein content.

Steward and Bidwell (1958) have proposed that there are two distinct types of proteins: (1) an "active" moiety, which is undergoing rapid breakdown and resynthesis and (2) the "inactive" moiety, which, once synthesized, is not reutilized or broken down. They further proposed that the growing cell as contrasted with the resting cell has its rate of metabolic turnover of the "active" protein complement very much accelerated, whereas, the protein of the resting cell remains much more in the quiescent condition.

MATERIALS AND METHODS

Flowers of snapdragon (Antirrhinum majus Linn.) were obtained from the horticulture greenhouse and used as the source of pollen. Fresh pollen was collected for each experiment. Germination tests showed that pollen collected shortly after anther dehiscence germinated best.

A nutrient medium containing 1 g. sucrose, 1.2 mg boric acid and 5.92 mg calcium nitrate in 10 ml medium was found most suitable for germination and pollen tube growth. The pH of the medium was about 6.7. The medium was prepared using deionized distilled water and analytical reagent grade chemicals. Pollen grains germinated in 45 min at 28°C.

Incorporation of ^{14}C-leucine

Preliminary experiments were performed to determine whether ^{14}C from labelled leucine was incorporated into the protein of pollen. Since these investigations revealed a greater uptake of leucine by the germinating pollen after 90 min incubation, as determined by radioactivity counts, data in all experiments were obtained after 90 min incubation unless otherwise specified.

Germination was carried out by the hanging drop technique by Goss (1962). ^{14}C-leucine (0.00013 mg/ml; specific activity 251 uC/uM, New England Chemical Corporation) was used at a concentration of 2 ml in 10 ml of growth medium. Eight cultures, 200 ml each, were suspended from the cover of a petri dish. The pollen was sprinkled over the nutrient medium and the cover carefully inverted over the bottom of the dish which contained a layer of water to maintain a high relative humidity. After inoculation, the dishes were placed in an incubator at 28°C for the required length of time.

Puromycin at a concentration of 50 ppm, was used in the nutrient medium

to investigate its effects on the incorporation of ^{14}C-leucine into pollen. Each treatment had five replications.

The petri dishes were removed from the incubator after the specified length of time and the pollen and nutrient medium were washed into a pyrex test tube. A 10 percent sucrose solution was used as a wash solution to minimize the rupturing of pollen grains. The tubes were centirufuged for 5 min at 1000 g. The supernatant liquid was poured off and collected in a test tube. The pollen was again washed with a small quantity of 10 percent sucrose solution and the tubes were centrifuged. The washings were collected in respective test tubes and saved for radioactivity counts. The volumes of these samples were measured.

The pollen was extracted overnight with 5 ml of 80 percent methanol at 4°C. The pollen proteins were precipitated out with an excess (1 ml.) of 5 percent trichloracetic acid. The test tubes, with methanol and the precipitated proteins and pollen grains, were centrifuged for 5 min at 1000g. The supernatant liquid was drawn off and collected in test tubes. The ppt was washed with a small quantity of deionized distilled water and centrifuged again. The washings were collected in respective test tubes and saved for radioactivity counts.

Determination of radioactivity

For total radioactivity counts, 2 ml. aliquots of each sample were transferred into metal planchettes. The samples were evaporated under a heat lamp. The amount of radioactivity added to the nutrient medium was determined for each experiment. Thus the amount of ^{14}C-leucine incorporated into the various fractions was estimated. The total amount of radioactive leucine incorporated was determined by measuring the activity in samples of

the supernatant and of the residue. Activities in each of these fractions were ascertained in a decimal scaler (Nuclear-Chicago). No correction for self absorption was made but the same amount of material was placed on each planchette in all experiments.

Protein hydrolysis and paper chromatographic separation of labelled compounds

The pollen residues, which were insoluble in alcohol, were hydrolyzed overnight in 6N HCl at 250°C. The hydrolysis was carried out in glass tubes, 72 x 10 mm. The protein and HCl were placed in the tubes and the tubes were then sealed in a propane-oxygen flame. The tubes were marked with a diamond pencil for further identification. On completion of hydrolysis, the tubes were cooled. The tubes were scored with a sharp file at a point below the sealed end and cracked. Fifty µl aliquots of the hydrolysate were spotted, using a micropipette, on a No. 1 Whatman chromatographic paper and developed one-dimensionally using butanol: formic acid: water (4:1:5) as a solvent for about 20 hours.

Each chromatogram was placed on blue brand, x-ray film (Eastman Kodak Co., Rochester, N.Y.). These were placed in a film holder with aluminum foil in between the films. All manipulations were done in the dark room using a Kodak 6-B light filter. The folders were left undisturbed for about 30 days. Films were then developed as recommended by the manufacturer.

Pollen germination and pollen tube growth at different levels of puromycin

To determine the effect of puromycin on germination and pollen tube growth, puromycin at concentrations of 0, 20, 40, 60, 80 and 100 ppm was added to the basic medium. Pollen were germinated by the aforesaid technique.

Ten replications of each treatment were set up. Photomicrographs of each replication were taken and germination percentage and pollen tube length were measured from the photographs.

Determination of total nitrogen and protein nitrogen in non-germinating and germinating pollen

Digestion. Freshly collected pollen were weighed and transferred to a pyrex test tube. The pollen were digested with 50 percent H_2SO_4 at 250°C for an hour. Completion of digestion was indicated by the appearance of a clear solution on the addition of four drops of 30 percent hydrogen peroxide to the cooled digest. The digest was heated for an hour and then diluted to 10 ml and 1 ml aliquots were used to determine total nitrogen. The experiments were conducted with 4 replications.

For nitrogen determination in germinating pollen grains, weighed amounts of pollen were germinated in the basic medium. The pollen grains and the nutrient medium were precipitated out with an excess of 5 percent TCA. The precipitated proteins were separated from the supernatant by centrifugation and digested with conc. H_2SO_4 (Sp. gr. 1:84). The acid digest was diluted to 10 ml as before and protein nitrogen was determined in 1 ml aliquots.

Estimation of nitrogen. The O'Brink modified Conway diffusion unit was used for the determination of nitrogen in all the samples. 0.025 percent NPX and 40 percent NaOH were used in the closing chamber to ensure sealing of the unit. A measured amount of boric acid solution with brom-cresol green indicator was placed in the center well. The acid digest and an excess of 40 percent NaOH were placed in the outer diffusion chamber without the two solutions being allowed to mix. The cover was placed on

the dish and the whole unit was carefully swirled to mix the digest and NaOH. The NH_3 released was absorbed by the boric acid. The diffusion was allowed to continue for three hours and the amount of ammonium borate formed was determined by titration with 0.005 N HCl.

RESULTS AND DISCUSSION

Formulation of nutrient medium for germination of Snapdragon pollen

Preliminary investigations were conducted to evolve a nutrient medium, suitably for the germination of snapdragon pollen. Sucrose for osmotic pressure, as a source of energy and as a carbon source and boron were presumed to be indispensable. The two critical factors studied for the purpose were (1) temperature required and (2) optimum concentration of $Ca(NO_3)_2$. Results obtained indicated that 2·969 gm/liter $Ca(NO_3)_2$ and 28°C temp were the most suitably for maximum germination and pollen tube growth. Accordingly the composition of the nutrient medium formulated for this study was as follows:

10 ml. of the medium contained

Sucrose.....1g

Boron.....1.2mg

$Ca(NO_3)_2$.....5.92mg

Total nitrogen and protein nitrogen determination in germinating and non-germinating pollen.

Data obtained are shown in Table 3. The total nitrogen and protein nitrogen are greater in germinating than in non-germinating pollen. Interpretations of these results is discussed later.

Incorporation of ^{14}C-leucine into pollen protein

It has been established that intact tissues of higher plants readily incorporate ^{14}C-labeled amino acids into their cellular proteins. A plant tissue incubated in the presence of a radioactive amino acid accumulates readioactivity in the cells. This method of study of protein synthesis has

been adopted in cell-free systems as well. When ^{14}C-leucine was added to the nutrient medium, little incorporation of ^{14}C into the pollen protein fraction was observed after thirty minutes incubation, but an increase in incorporation of ^{14}C was attained after ninety minutes. Oaks (1965) reported a similar time lag in the incorporation of exogenously fed leucine-^{14}C to maize root tips. According to Britten and McClure (1962), this lag represented the time required to saturate the leucine pool that is the precursor for protein.

Uptake and loss of radioactivity

Table 1 shows data on the residual radioactivity in the external solution and the amount of radioactivity which remained in the pollen. Total radioactivity incorporated into the protein fraction was in all cases less than 30 percent of the radioactivity supplied. Appreciable losses seem to have occurred during extraction procedures. Another source of such loss may be the respired CO_2.

It is well known that every protein requires certain amino acids for its synthesis. If any one of these is lacking, that particular protein is not synthesized regardless of the fact that the other amino acids are supplied in surplus quantities. This fact has been stressed in human nutrition (Jones 1939). Studies on the effects of such amino acid imbalance in animals have been reviewed by Elvehjam (1956). Although the optimum ratio may differ with the species under study, it is considered that the high level of one amino acid may be detrimental to the utilization of other amino acids.

It may be suggested that high levels of exogenously supplied leucine were more than that required by the pollen and could not, therefore, be

totally utilized. The fraction of ^{14}C-leucine that was not utilized in protein synthesis may have been given off as CO_2 as a consequence of deamination and oxidation. In such a case the fate of the nitrogen released needs to be explained.

Oaks (1965) reports 100 percent recovery of ^{14}C-U-leucine supplied to maize roots tips. This variance in the percentage of recovery of radioactivity is conceivable as being due to the fact that utilization of the exogenous amino acid is dependent on the free amino acids present. The amounts of such amino acids should be considerably less in a single cell than in a group of cells.

Autoradiographs of labelled components after protein hydrolysis

No spots developed on films in any of the treatments. This may be due to the extremely low concentration of ^{14}C-leucine used.

The influence of puromycin on incorporation of ^{14}C-leucine into protein and on germination percentage and pollen tube growth

Many lines of evidence support the view that RNA and protein synthesis may function during pollen tube growth. Developments in the field of protein and nucleic acid biochemistry provide a background of information on specific inhibitors of protein synthesis, which facilitate the study of RNA and protein synthesis. Puromycin is an effective inhibitor of protein synthesis and was, therefore, employed in the present study.

From the data presented in Table 1, it is evident that puromycin at a concentration of 50 ppm exhibited no inhibitory effect on the incorporation of ^{14}C-leucine. On the other hand, the incorporation of ^{14}C-leucine, as evidenced by radioactivity counts, increased almost two-fold as compared to treatment without puromycin under similar conditions of exposure.

The data obtained on the effects of different levels of puromycin on germination and pollen tube growth (Table 2) indicate that values for both germination percentage and pollen tube length show statistically significant results at 40 and 60 ppm concentration. At 80 ppm the germination percentage is less than 20 percent of the control, while pollen tube length is unaffected. At 100 ppm both values are significantly higher indicating a clear stimulation in both phenomena. Sen (1960) also obtained a stimulation of pollen tube growth in Corchorus olitorius with penicillin and streptomycin, although growth rates varied with different concentrations of the two substances.

Incorporation of ^{14}C-leucine into the protein fraction of pollen with or without puromycin and the absence of inhibitory effect of puromycin on pollen tube growth suggest two possibilities, namely (1) that the membranes of the pollen grains are not permeable to puromycin or (2) that there is an alternative pathway of protein synthesis which is not inhibited by puromycin. If the first hypothesis is valid, then puromycin did not reach the site of protein synthesis and therefore did not inhibit such synthesis. The second hypothesis finds support in the conclusion of Stanley et al. (1958) and Tupy (1963, 1964, 1966). Increase in total and protein nitrogen (Table 3) also supports such an assumption.

Experiments with radioactive amino acids on growing cells show an incorporation of these into the protein fraction of the cell suggesting a synthesis of protein during cell growth (Steward and Bidwell 1958). Incorporation of tritiated nucleosides into the nuclei of pollen indicates that there is RNA and DNA synthesis or turnover occurring in the growing pollen tubes (Young and Stanley 1963). The protein formed in the developing pollen was found to be directly proportional to its RNA content (Woodward 1958). Data on protein nitrogen changes however have been interpreted as indicating that protein synthesis does not occur during the growth of the tube (Poszar

Table 1:

UTILIZATION OF LEUCINE BY SNAPDRAGON POLLEN*

	Without Puromycin				With Puromycin	
	30 minutes incubation		90 minutes incubation		90 minutes incubation	
	CPM	% distribution of ^{14}C recovered	CPM	% distribution of ^{14}C recovered	CPM	% distribution of ^{14}C recovered
Initial	63.8		93.2		65.8	
Methanol- soluble	0.33	0.517	0.44	0.472	Negligible.....	
Residue	2.1	3.29	9.5	10.19	17.33	26.33
Medium after germination	2.99	4.686	2.3	2.467	2.15	3.27
Total recovered	5.43	8.493	12.24	13.129	19.48	29.60

LSD at 5% = 4.0680)
) for residue
LSD at 1% = 5.8500)

* Leucine-^{14}C (24.9 µc with specific activity = 251 µc/µM) was present in the germinating medium.

Table 2: Effect of puromycin on germination percentage and tube development of snapdragon pollen (after 45 minutes at 28°C).

Conc. ppm Puromycin	Germination % (Av. of 7 rep.)	Pollen tube length (μ) (Av. of 7 rep.)
0	42	167
20	42.1	166
40	20	132
60	23	106
80	9.1	167
100	59.1	210

LSD for germination % at 5%--14.42

LSD for pollen tube length at 5%--5.76

Table 3:

Total and protein nitrogen content

of snapdragon pollen

	Before germination (as percent)	After germination (as percent)
Total nitrogen	1.86	2.78
Protein nitrogen	0.55 (estimated)	0.865

1960). This view would assume that the growth of the tube is caused either by the absorption of water and/or by turnover of protein. Results of radioisotope studies, however, indicate that there is a net synthesis of protein rather than a mere turnover (Stanley, Young and Graham 1958; Tupy 1966).

The current concept of protein synthesis assumes the involvement of all three RNA's, namely m-RNA, s-RNA and r-RNA in protein synthesis. Protein synthesis is known to be a corollary process with the growth of a cell. Reports of studies with such specific inhibitors of protein synthesis as Actinomycin-D and 5-flourouracil indicate that fifty percent RNA synthesis was inhibited without affecting growth (Perry 1964; Aronson 1961; Key and Ingle 1964). These results suggest that the synthesis of new r-RNA and s-RNA are not necessary for cell elongation. Key and Ingle (1964) also found that these two substances strongly inhibited the synthesis of s-RNA and r-RNA but that these two substances strongly inhibited the synthesis of s-RNA and r-RNA but that of m-RNA was only slightly impaired, indicating a requirement for only m-RNA synthesis for the growth of the tissue. These results cumulatively suggest that alternate pathways to protein synthesis may exist in plant cells, although not of very common occurrence. Such a deviation from the general pattern of protein synthesis has been reported in sieve tubes and germinating cotton seeds.

The growth stimulating effects in pollen, of base analogues, which are antimetabolites of nucleic acids, have been reported (Tupy 1964). That the base analogues act partly by their participation in nucleic acid synthesis is also fairly well established (Tupy 1966). Their stimulatory effects on pollen may be considered analogous to that of 5-fluorouracil on soybean hypocotyl (Key and Ingle 1964). It may be suggested, therefore,

that the primary species of RNA that is synthesized in the pollen tube, on which, in turn, protein synthesis depends, is m-RNA. It is hypothesized that protein synthesis in pollen is dependent on such m-RNA (Tupy 1966) and that the m-RNA is the stable, long-lived type (Mascarenhas 1966; Tupy 1966).

Puromycin is known to inhibit the transfer of amino acids from s-RNA to r-RNA, but not the formation of aminoacyl RNA. The specific blockage, therefore, occurs at a point beyond the synthesis of m-RNA, which is formed on DNA. The absence of inhibitory effects of puromycin, as interpreted by the incorporation of ^{14}C-leucine into pollen protein and pollen tube growth, may be explained as being due to a continual synthesis of protein via m-RNA, the synthesis of which is not disturbed by puromycin.

The time of such m-RNA synthesis is unknown. It has been suggested that m-RNA is synthesized before the pollen is released from the anthers. Confirmation of such a hypothesis should be sought in long term experiments.

SUMMARY

Studies on *in vitro* protein synthesis in snapdragon pollen were made. ^{14}C-leucine was used to determine protein synthesis during pollen tube growth. The effects of puromycin on such synthesis and pollen tube growth were also studied. The total and protein nitrogen contents of non-germinating and germinating pollen were estimated.

Radioactivity counts indicate an incorporation of ^{14}C-leucine into pollen proteins. Puromycin caused an increase in such incorporation. Data on pollen germination and tube growth indicate a stimulation of both phenomena by puromycin at 100 ppm. Total nitrogen and protein nitrogen increased in germinating pollen as compared to non-germinating pollen. These results have been interpreted as indications of an occurrence of protein synthesis *in vitro* during pollen tube growth. An alternative mechanism of protein synthesis, hypothesized by other investigators, has been suggested for snapdragon pollen.

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LITERATURE CITED

*Amici, G. B. 1824. Observations Microscopiques Sur diverses especes de plantes. Ann. Sci. Nat. Bot. 2:41-70, 211-248

*_____. 1830. Note sur le mode d'action du pollen sur le stigmate. Extrait. d'une lettre d'Amici a'Mirabel. Ann. Sci. Nat. Bot. 21: 329-332.

Aronson, A. I. 1961. The effect of 5-flourouracil on bacterial protein and ribonucleic acid synthesis. Biochem. Biophys. Acta. 49:98-107.

Bathurst, N. O. 1954. The amino acids of grass pollen. J. Expt. Bot. 5: 253-256.

Blank, F. and Frey-Wyssling, A. 1941. Protoplasmawachstum und stickstoff wanderung in der koleoptile von Zea mays. Ber. Schweiz. Bot. Ges. 51:116-142.

Brewbaker, J. L. and Emery, G. C. 1961. Pollen radiobotany. Rad. Bot. 1: 101-154.

Brink, R. A. 1924. The physiology of pollen. I. The requirements for growth. Amer. J. Bot. 11:218-228.

Britikov, E. A., S. V. Vladimirtseva and Musatova, N. A. 1965. Transformation of proline in germinating pollen and pistil tissues. Fiziol. Rast. 12(6):953-967.

Britikov, E. A., N. A. Musatova and S. V. Vladimirtseva. 1966. Effect of proline and its antimetabolites on pollen germination and pollen growth Antirrhinum majus, Salix caprea, Petunia hybrida, Transdescantia virginiana. Fiziol. Rast 13(6):978-987.

Britten, R. J. and F. T. McClure. 1962. The amino acid pool in Escherichia coli. Bacteriol. Rev. 26:292-335.

Brown, R. and Broadbent, D. 1950. The development of cells in the growing zones of the root. J. Expt. Bot. 1:249-263.

Bryan, J. M. D. 1951. Deoxypentose nucleic acid (DNA)-protein relations during microsporogenesis of Tradescantia, Chromosoma, 4:369-392.

Callaghan, O. H. and A. R. Goldfarb. 1962. Isolation and properites of an allergen from dwarf ragweed pollen. J. Immunol. 89:612-622.

Chang, C. W. and A. Kivilaan. 1962. Studies on the nucleic acids of Oenothera organensis pollen. Plant Physiol. 37(Suppl):21.

Chauvshina, N. P. and Melhikov, V. K. 1964. Physiological and biochemical characteristics of sterile pollen from distant hybrids of fruit and berry plants. Fiziol. Rast. 11(2):330-333.

*Cazpek, F. 1920. Biochemie der Pflanzen 2: Gustav Fischer. Jena.

Dure, L. and L. Waters. 1965. Long-lived messenger RNA:evidence from cotton seed germination. Science 147:410-412.

East, E. M. 1929. Self-sterility. Biblio. Genet. Lpz. 5:331-370.

Elvehjam, C. A. 1956. The effects of amino acid imbalance on maintenance and growth. Ann. Conf. on protein metabolism. Rutgers University, New Brunswick, N. J. 22-30.

Franke, W., M. Liesalotte and K. Heye. 1965. The so called glucose oxidase. VIII. The effect of a variation in the carbon and nitrogen sources on induced enzyme formation in Aspergillus niger. Arkh. Mikrobiol. 51: 323-350.

Frey-Wyssling, A. and Blank, F. 1948. The growth in surface of the plant cell wall. Eighth symposium on development and growth. Growth. 12 (Suppl):151-169.

Frey-Wyssling, A. and F. Blank. 1940. Growth of protoplasm and nitrogen migration in the coleoptile of Zea mays. Nature, 145:974-975.

Goss, J. A. 1962. In vitro germination of Ornithogalum audatum Ait. pollen. Kans. Acad. Sci. 65(3):310-317.

Gross, P. R., Malkin, L. A. and Moyer, W. A. 1964. Templates for the first proteins of embryonic development. Proc. Natol. Acad. Sci. U. S. 51:407-414.

Grover, R. K. and J. S. Sidhu. 1965. Effect of nitrogen sources on the growth of Pythium aphanidermatum (Edson). Fitz. Syndowia Ann. Mycol. 19(1/6):231-237.

Harvey, H. W. 1953. Synthesis of organic nitrogen and chlorophyll by Nitzchia closterium. J. Marine Biol. Assoc. United Kingdom. 31(3): 477-487.

Heyl, F. W. 1917. Analysis of ragweed pollen. J. Amer. Chem Soc. 39:1470-1476.

_____. 1919. The protein content of ragweed pollen. J. Amer. Chem. Soc. 42:1470-1476. 41:670-682.

_____. and H. H. Hopkins. 1920. The ragweed pollen. J. Amer. Chem. Soc. 42:1738-1744.

Johri, B. M. and I. K. Vasil. 1961. Physiology of pollen. Bot. Rev. 27: 325-381.

Jones, D. B. 1939. Protein requirement of man. Food and life. U.S.D.A. Yearbook of Agriculture.

Kanshina, M. V. 1964. Correlation between the amino acid content in the pollen and pistils of cherry plants and winter resistance of the flower buds. Fiziol. Rast 11(5):920-921.

Key, J. L. 1964. Ribonucleic acid and protein synthesis as essential processes for cell elongation. Plant Physiol. 39:365-370.

Key, J. L. and J. Ingle. 1964. Requirement for the synthesis of DNA-like RNA for growth of excised plant tissue. Proc. Natl. Acad. Sci. U.S. 52:1382-1388.

Khoo, U. and H. T. Stinson (Jr.). 1957. Free amino acid differences between cytoplasmic male sterile and normal fertile anthers. Proc. Natl. Acad. Sci. 43(7):603-607.

*Kopp, M. 1948. Uber das Sauerstoffbedurfnis wachsender Pflanzerzellen Ber. Schweiz. Bot. Ges. 58:283-318.

Larson, D. A. 1965. Fine structural changes in the cytoplasm of germinating pollen. Amer. J. Bot. 52:139-154.

Lensu, L. V. 1963. Effect of nitrogen containing compounds on the germination of pollen. Uch. Zap. Leningr. Gos. Ped. Inst. 239:139-142.

Lewis, D., S. Burrage and D. Walls. 1967. Immunological reactions of single pollen grains, electrophoresis and enzymology of pollen protein exudates. J. Expt. Bot. 18(55):371-378.

*Lidforss, B. 1896. Zur. Biologie des Pollens. J. Wiss. Bot. 29:1-38.

Linskens, H. F. 1958. Physiological studies of ripening. II. Changes in the content of nucleic acids during the meiosis of pollen and development of pollen in Lilium henryi. Acta Botan. Neerl. 7:61-68.

Linskens, H. F. 1966. Variations in the protein and enzyme pattern during pollen meiosis and pollen development. Planta. 69(1):79-91.

Lubliner-Mianowska, K. 1956. Investigations on the chemical composition of pollen I. The study of pollen as bee food. Acta. Soc. Botan. Polon. 25:409-419.

Makinen, Y. and J. L. Brewbaker. 1967. Isoenzyme polymorphism in flowering plants I. Diffusion of enzymes out of intact pollen grains. Physiol. Plant. 20(2):477-482.

Mascarenhas, J. P. 1966. Pollen tube growth and ribonucleic acid synthesis by vegetative and generative nucleic of Tradescentia. Amer. J. Bot. 53(6):563-569.

Mathaei, H. 1957. Comparative studies of the protein content during the growth by elongation of the petals and other tissues. Planta 48: 468-522.

Moses, M. J. and J. H. Taylor. 1955. Deoxypentose nucleic acid synthesis during microsporogenesis in Tradescantia. Exptl. Cell Res. 9:474-488.

Nasatir, M., A. M. Bryan and S. D. Rodenberg. (1960). Changes in soluble proteins of developing lily anther. Science 132:897-898.

Nielsen, N., J. Grommer and R. Lunden. 1955. Investigations on the chemical composition of pollen from some plants. Acta Chem. Scand. 9:1100-1106.

Nooden, L. D. and K. V. Thimann. 1963. Evidence for a requirement for protein synthesis for auxin induced cell enlargement. Proc. Natl. Acad. Sci. U. S. 50:195-200.

_____. 1965. Inhibition of protein synthesis and of auxin induced growth by Chloramphenicol. Plant Physiol. 40: 193-201.

Oaks, A. 1965. The effect of leucine on the biosynthesis of leucine in maize root tips. Plant Physiol. 40:149-155.

Palilova, A. N. and V. K. Hensman. 1964. Cytochemical study of male sterility in corn. Vopr. Genet. i Selektsii. 85-94.

Perry, R. P. 1964. Selective effects of Actinomycin D on the intracellular distribution of RNA synthesis in tissue culture cells. Exptl. Cell Res. 29:400-406.

*Planta, A. Von. 1884. Uber die chemische Zusammensetzung des Blutherstaubes der Haselstaude. Landw. Versuchsstar. 31:97-101.

*Planta, A. Von. 1886. Uber die chemische Zusammensetzung des Bluthenstaubes der gameinen Kiefer (Pinus sylvestris). Lands. Versuchsstat. 32:215-219.

Poddubnaya - Arnoldi, V. A., N. V. Tsinger, T. P. Petrovskaya and Polunina. 1959. Histochemical study of the pollen grains and pollen tubes in the angiosperms. Recent Adv. Bot. 1:682-685.

Poddubnaya - Arnoldi, V. A., N. V. Tsinger, T. P. Petrovskaya and N. N. Polunina. 1961. Histochemical investigations of pollen and pollen tubes in some angiospermous plants. Tr. Gl. Botan. Sada. Akad. Nuak. SSSR. 8:162-194.

Poszar, B. I. 1960. The nitrogen metabolism of the pollen tube and its function in fertilization. Acta Botan. Acad. Sci. Hung. 6:389-395.

Revel, M. and H. H. Hiatt. 1964. The stability of liver messenger RNA. Proc. Nat. Acad. Sci. U. S. 51:810-818.

Rodkiewicz, B. 1960. Determination of deoxyribonucleic acid by Feulgen-photometry in the nuclei of pollen grain of Tradescantia bracteata. Acta. Soc. Botan. Polon. 29:211-217.

Sarkar, B. C. R., S. H. Wittwer, R. W. Luecke and H. M. Sell. 1949. Quantitative estimation of some amino acids in sweet corn pollen. Arch. Biochem. 22:353-356.

Sato, S. 1964. Variation of DNA amount during differentiation of the nuclei of pollen of Tradescantia. Sci. Dept. Fac. Lit. Sci., Hirosaki Univ. 11(1):35-39.

Sawada, Y. 1958. Physiological and morphological studies on pollen grains. VII. Effects of amino acids on germination of pollen grains and on growth of pollen tubes. Botan. Mag. (Tokyo) 71:218-223.

Scott, R. B. and B. Bell. 1964. Protein synthesis during development: control through messenger RNA. Science 145:711-713.

Schon, A. H. and M. Richter. 1959. Studies on ragweed pollen IV. Isolation and characterization of an allergenic fraction from ragweed pollen. J. Immunol. 82:190-197.

Sen, S. K. 1960. On the effects of antibiotics (penicillin and streptomycim) on the rate of pollen tube growth in Corchorus olitorius L., strain C. G., in vitro. Indian Agr. 4:59-62.

Shpakova, V. M. 1961. Biochemical investigation of vine pollen in connection with later fertilization. Sadovodstvo' Vinogradarstvo i Vinodelie Moldavii 16(6):26-28.

Simola, L. K. 1967. The effect of some non-protein amino acids on pollen germination and pollen tube growth in five species of Vicieae. Planta 77(4):287-297.

Stanley, R. G. and H. F. Linskens. 1965. Protein diffusion from germinating pollen Physiol. Plant. 18(1):47-53.

Stanley, R. G., L. C. T. Young and J. S. D. Graham. 1958. Carbon dioxide fixation in germinating pine pollen (Pinus ponderosa). Nature 182:1462-1463.

Steward, F. C. and R. G. S. Bidwell. 1958. Nitrogen Metabolism, Respiration and growth of cultured plant tissue IV. The impact of growth on protein metabolism and respiration of carrot tissue explants. J. Exp. Bot. 9(26):285-305.

Steward, F. C., J. F. Thompson and J. K. Pollard. 1958. Contrasts in the nitrogenous composition of rapidly growing and non-growing plant tissues. J. Expt. Bot. 9(25):1-10.

Tano, S. and H. Takahashi. 1964. Nucleic acid synthesis in growing pollen tubes. J. Biochem. (Tokyo). 56:578-580.

Tsinger, N. V. 1961. Biochemical evolution of pollen in angiospermous plants. Tr. Gl. Botan. Sada, Akad. Nauk SSSR. 8:149-161.

Tulecke, W. 1960. Arginine requiring strains of tissue obtained from Ginkgo pollen. Plant Physiol. 35:19-24.

Tulecke, W. and A. Rutner. 1965. Changes in the amino acid composition of medium and cells of a plant tissue culture during growth in a liquid medium containing arginine. Proc. Intern. Conf. Plant Tissue cult. Penna. State Univ. 103-116.

Tupy, J. 1961. Investigations of free amino acids in cross-self, and non-pollinated pistils of Nicotiana alata. Biol. Plant. 3:47-64.

Tupy, J. 1963. Changes in pollen proteins during tube growth from incompatibility point of view. Genetics Today 1:212-213.

Tupy, J. 1964. Metabolism of proline in styles and pollen tubes of Nicotiana alata p. 86-94. In H. F. Linskens (ed.) Pollen physiology and fertilization. North-Holland Publ. Co., Amsterdam.

Tupy, J. 1966. Synthesis of protein and RNA in pollen tubes stimulated with 2-Thiouracil. Biol. Plant 8(5):398-410.

Turpin, N. V., V. E. Bormotov, V. K. Savchenko and I. V. Matoshko. 1965. Content of nucleic acids in the pollen of diploidal and tetraploidal beet. Doke. Akad. Nauk. SSSR. 161(3):715-717.

Woodward, J. W. 1958. Intracellular amounts of nucleic acids and protein during pollen grain growth in Tradescantia. J. Biophys. Biochem. Cytol. 4:383-390.

Young, L. C. T. and R. G. Stanley. 1963. Incorporation of tritiated nucleosides, thymidine, uridine and cytidine in nuclei of germinating pine pollen. The Nucleus, 6:83-90.

*Originals not seen.

NITROGEN METABOLISM IN POLLEN

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AN ABSTRACT OF

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ABSTRACT

The occurrence of protein synthesis during germination and pollen tube growth has been debated for sometime. The present investigation was undertaken to study protein synthesis during pollen tube development and the effect of puromycin on such synthesis.

Freshly collected pollen from snapdragon flowers were germinated by the hanging drop technique. The nutrient medium evolved for this study contained 1 g sucrose, 1·2 mg boron and 5·92 mg $Ca(NO_3)_2$ in 10 ml. Leucine -^{14}C was added to the medium. In the study involving puromycin, the antibiotic was added in desired concentrations to the nutrient medium.

The pollen was incubated for a specified time and the residue separated from the supernatant liquid. The nutrient medium after germination, thus separated formed the first fraction on which radioactivity counts were taken. The pollen was extracted with 80 percent methanol overnight and separated from the methanol soluble compounds. These two fractions were then used for radioactivity determinations. Radioactivity counts were made on aliquots of the nutrient medium and methanol fractions. Incorporation of ^{14}C-leucine was obtained in all three treatments.

The protein so obtained was hydrolyzed and the products of hydrolysis were chromatogrammed one dimensionally. The chromatograms were placed on x-ray films which were developed after about 30 days. Autoradiographs did not reveal any spots. Low concentration of ^{14}C-leucine used in the medium may be the cause of failure to detect radioactivity.

Pollen germination and tube growth was studied at concentrations of 0, 20, 40, 60, 80 and 100 ppm of puromycin. Data on germination percentage and pollen tube growth were obtained from photomicrographs. Results of this

study indicate a stimulation of germination and pollen tube growth at the highest concentration.

Changes in total and protein nitrogen content in germinating pollen were determined by the Kjeldahl's method. Estimations of total nitrogen content in non-germinating and germinating pollen indicated an increase during germination and pollen tube growth. The pollen proteins in germinating pollen were precipitated with 5 percent trichloroacetic acid and nitrogen content in the precipitate was determined. Since the ratio of protein nitrogen to total nitrogen would be the same in non-germinating as well as germinating pollen, the protein nitrogen content of non-germinating pollen was estimated from the ratio obtained for germinating pollen. The protein nitrogen values showed an increase in germinating pollen. Results obtained are discussed.