KEY

PLANT PHYSIOLOGY

First Hour Exam – Spring, 2006

1. Since you have mastered the intricacies of water relations in plants, please explain to my cousin from Kansas City <u>how</u> the Ψ of the soil and the Ψ and S of the root tissue will change and <u>why</u> when: (use **more positive** or **more negative** to indicate direction of changes)

   a) severe drought hits the Midwest during the summer of 2005

   Ψ of the soil will become more negative as water is lost from soil

   Ψ of the root will become more negative since less water is available to enter from the soil and water is transpired from the plant

   S of the root will be come more negative because there is less water in cells to dilute solutes

   b) flood waters from the great rains of 1993 wash over the fields of Iowa

   Ψ of the soil will become more positive because the soil solution will be diluted by rain

   Ψ of the roots will be come more positive because water is more available from the soil solution

   S of the roots will become more positive because solutes will be diluted by the entering water

   c) a poor unsuspecting corn plant suddenly finds itself growing in the Nevada Salt flats

   Ψ of the soil will become more negative because of higher solute concentration in the soil

   Ψ of the roots will become more negative as water leaves root tissue in response to lower Ψ of soil
   S of the roots will become more negative as water leaves the roots to enter the soil in response to a steep Ψ gradient thus concentrating the solutes in cells

2. I have hired you to treat the weeds in my lawn with the herbicide, 2,4-D, (2,4-dichlorophenoxyacetic acid). The herbicide must enter the cells to have an effect. You have a choice of two different carriers (solutions of inert ingredients) in which to dissolve the 2,4-D in before spraying it on my weeds. Carrier A has a pH of 7.5 and Carrier B of 4.5. Which carrier would you choose and why? Explain in detail.

Carrier B with a pH of 4.5 would be much more effective for treating the weeds because this would cause the 2,4-D (an acid) to be largely in its molecular form. In its molecular form, the 2,4-D is uncharged and thus able to pass through the cuticle and plasma membranes of the leaf cells to exert its effect.

3. Provide the following information regarding the vital staining experiment we did in lab if an **acidic dye** had been used instead of a basic dye. Color of molecular form = yellow, color of ionic form + red.

Indicate staining with (+) or (-)

	Color of solution	Wall staining	Vacuole staining	Color of vacuole
Solution 1 (pH 3.3)	**yellow**	**(-)**	**(-) or yellow**	**unstained or yellow**
Solution 7 (pH 8.1)	**red**	**(-)**	**(-)**	**colorless**

Indicate the color of each layer if you used the same acidic dye as above.

Solution 1		Solution 7
clear or light pink	water	**red**
yellow	chloroform	**clear or pale yellow**

What general principles of permeability does this experiment illustrate?

Charged, polar molecules cannot readily enter a cell across the plasma membrane; unstained apolar molecules can because of their greater llipid solubility

4. A strange new plant species was discovered by the Voyager space mission on the planet Jupiter. When ion uptake studies were conducted on its root system, it was found to have a primary active transport mechanism that differed from any previously known. The primary active transport mechanism in the plasmalemma used ATP to pump Na+ ions into the cytoplasm creating a steep sodium gradient. Thus it was a Na+-translocating ATPase. Answer the following questions concerning ion transport in this very unusual plant:

a) What will be the direction of the electrical gradient generated across the plasmalemma? (where more positive and where more negative)

More positive inside the cell than outside

b) What would be the most likely uptake mechanism used by this plant to move the following ions into or out of the cell? Please give a complete verbal description of each including what energy source is driving each transport mechanism.

To move K+ ions in -

An antiport with Na^+ moving out down its concentration gradient while K^+ move in -- Na^+ gradient is the energy source

To move Cl- ions in -

Facilitated diffusion of Cl^- down the electrical gradient – electrical gradient is source of energy using an ion channel

To move large uncharged polar solutes in -

Antiport with Na^+ moving out down its concentration gradient while solutes move in – energy from Na^+ gradient

To move anions out - **A symport of Na^+ and the anions moving outward driven by energy of Na^+ concentration gradient**

What would be the effect on the movement of ions across the plasmalemma if the ionophore Gramicidin A (a pore-former that transports cations with one charge) was added to the medium surrounding your root tissue?

The Na^+ gradient that is used to drive all the other transport mechanisms would be lost and all other selective ion transport would cease

5. You conducted an experiment to determine the amount of phosphate absorbed by aged potato disks. The following results were obtained for the experimental conditions listed below:

	mμmoles PO_4/gfw/hour
Control	6.0
+KCN	2.8
+DNP	2.6
+0° C	0.2

Describe the transport mechanism used by plant cells to absorb phosphate.

Phosphate enters the cell via a co-transport mechanism with H^+ from previously formed H^+ gradient produced by outwardly directed H^+-translocating ATPase

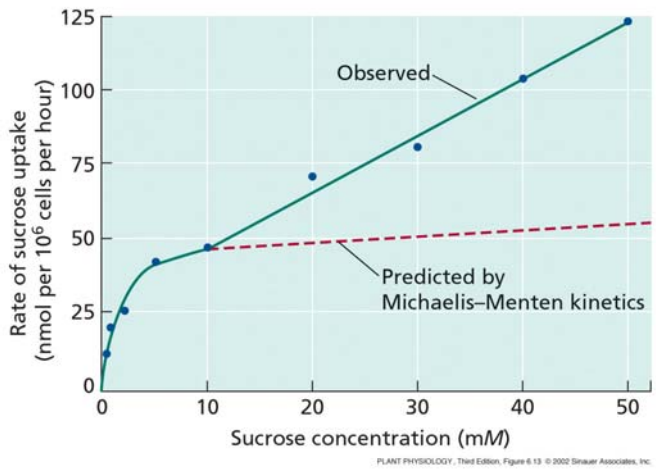
Explain **in detail** the physiological basis for the effect of potassium cyanide (KCN), dinitrophenol (DNP) and cold on the rate of uptake of phosphate in the experiment above.

KCN blocks electron transport in the mitochondria which prevents ATP synthesis. Lack of ATP prevents the H^+-translocating ATPase from creating the H^+ gradient necessary for phosphate transport

DNP uncouples electron transport from ATP synthesis, cutting off the supply of ATP with the same effect as CN

Cold temperatures slow down enzymatic reactions of the TCA cycle and glycolysis, interfering with the supply of NADH for the electron transport chain (ETC). The ETC then doesn't create the H^+ gradient needed to synthesize ATP for fueling H^+-translocating ATPases which create the H^+ gradient required for co-transport of phosphate

6. Describe the type of uptake processes that would most likely be operating for absorption of solutes from solutions of low (0-10mM) and high (10-50 mM) concentrations as illustrated in the graph below:



At low concentrations the likely uptake process is secondary active transport utilizing the energy of an existing proton gradient to move the sucrose into the cell with a carrier protein operating as a symport. This uptake has classic Michaelis-Menten kinetics showing saturation of carriers at higher concentrations and can be inhibited by metabolic poisons that cut off the supply of ATP

Uptake from high concentrations (10-50 millimolar) is thought to occur through carrier proteins in a process of facilitated diffusion. This uptake would not saturate at higher concentrations as seen in the graph and is not inhibited by metabolic poisons.

7. What did Mouline, et al. find to be the role of the SPIK protein in plant development? What evidence did they find from their studies of the spik-1 mutant that supported their conclusions about its role?

The SPIK protein appears to be a Shaker type inwardly directed K^+ channel that supports cell growth in pollen grains by supplying ions for maintaining turgor relations. This finding was supported by the electrophysiological studies that showed a strong inward K^+ current when functional SPIK protein was present but was missing in the spik-1 mutant. This was also supported by studies comparing wild type and spik-1 pollen grains with regard to pollen germination and pollen tube growth, both of which were impaired in the mutant. They also showed that the mutant pollen had impaired pollen competitive ability.

8. Describe how the enzyme-linked immunoassay that you used in lab to detect the hormone abscisic acid works.

The assay uses the competitive antibody binding method to measure concentration of ABA. Each assay well is coated with monoclonal antibodies to ABA. You add equal amounts of the plant extract containing ABA and of ABA-linked alkaline phosphatase (tracer). A competitive binding reaction is set up between the constant amount of tracer and the unknown amount of ABA in the plant extract. The hormone in the extract competes with the tracer for antibody binding sites. The unbound tracer is washed away before adding a substrate that turns yellow when the alkaline phosphatase on the tracer reacts with it. Thus the yellow color produced when the enzyme of the tracer reacts with the substrate is inversely proportional to the amount of the hormone in the sample. In other words the more color produced in the well, the less ABA there was in the sample.

Which of the following wells will have the highest absorbance, one containing 100 pmoles of ABA or 10 pmoles of ABA? Explain.

The well containing 100 pmoles of ABA will have the lowest absorbance (smallest amount of yellow) because the ABA outcompeted the tracer for the antibody binding sites.

The 10 pmole well will have the highest absorbance because there was much less ABA to compete for the binding sites.

What did your results show regarding the relative effect of osmotic stress versus cold and heat stress? In other words, which stress seemed to be stronger?

The osmotic stress seemed to be stronger than the cold stress since it induced the production of more ABA based on the amounts measured in the ELISA assay