Give succinct, precise answers in the space provided after each question. Unless otherwise noted in the margin each section of each question is worth 4 points.

1. (a) An important product of the wound-induced signal transduction chain is protease inhibitor. How could this product confer some protection for the plant against what was inflicting the wound?
   Ans.: Protease inhibitor (PI) interferes with the ability of the herbivore to digest the plant protein. By reducing the nutrition the herbivore can receive from the plant, the PI reduces its growth and fecundity.

(b) What is an enzymatic step that occurs after the production of systemin that leads to the production of jasmonic acid? Your answer should name the enzyme, the substrate and the product.
   Ans.: Lipase generates fatty acids (especially linolenic acid) from phospholipids; lipoxigenase converts linolenic acid to JA.

(c) Describe evidence that the enzyme activity noted in your answer to 1b is needed for plant wound defense?
   Ans.: Plants depressed in lipase or lipoxigenase activity produce less proteinase inhibitor in response to wounding.

2. (a) What are two wound-induced changes that are blocked by suppression of MPK1 synthesis?
   Ans.: Production of protease inhibitors and production of jasmonic acid are blocked.

(b) In the assay of MPK1 activity, what is the role of MBP (myelin basic protein)
   Ans.: It is used as the protein substrate for MPK1, and its phosphorylation can be visualized by $^{32}$P from $^{32}$P-ATP.

(c) The Figure below shows % survival of mutant or WT plants that are attacked by gnat larvae. Three different plots of % survival are described. State what mutant was used for this experiment, and for each plot, describe on the line provided what plant (mutant or WT) was sprayed and with what it was sprayed.

3. (a) There is evidence that JA needs to be modified to be active. What is the modification, and what is the evidence it is needed?
   Ans.: JA is activated by being conjugated to Isoleucine. If the enzyme that catalyzes this conjugation is knocked out, then JA responses in plants are severely diminished.

(b) What are the roles of JAZ proteins and MYC2 in JA signaling, and how does JA alter their level or activity?
   Ans.: JAZ proteins are repressors of the transcription of MYC2, which is a positive regulator of the transcription of genes turned on by JA. JA treatment results in ubiquitination and proteolysis of JAZ proteins and this increases the transcriptional activity of MYC2.

(c) The NH3 level rises in the digestive tract of M. sexta larvae that feed on WT plants. What activity accounts for this rise, and why can this activity serve as an effective plant defense strategy?
   Ans.: Feeding (wound) stimulus increases JA levels, which induce higher levels of threonine deaminase in plants. When M. sexta larvae ingest this enzyme it catalyzes the removal of amine groups from threonine, in their digestive tract thus causing the NH3 level to rise there.
4. (a) Studies of plant-animal communication revealed one reason why many American varieties of corn are more susceptible to damage from grubs that feed on corn roots than many European varieties. Explain.

**Ans.** Some American varieties of corn, unlike some European varieties, cannot make caryophyllene in response to grub feeding on their roots, and since caryophyllene can attract parasitoid worms to kill the grubs, the American varieties that do not have this defense response are more susceptible to root damage from grubs than the European varieties that can make caryophyllene in response to grub attacks.

(b) The plant volatiles induced by volicitin are interpreted by two different animals differently. Explain.

**Ans.** Parasitoid wasps are attracted to plants by the plant volatiles that are induced by volicitin, but the moths that lay the eggs that hatch into army worms are repelled by these same volatile signals.

(c) In the olfactometer used to assay plant-animal communication in roots, what was placed in the central cylinder, and what was measured after adding this to the central cylinder?

**Ans.** Parasitoid worms that are attracted by caryophyllene were added to the central cylinder, and what was measured was the relative number of worms that moved from the cylinder toward peripheral ports that contained caryophyllene or wounded plants that could make caryophyllene, compared to the number that moved toward ports that were empty or contained unwounded plants.

5. (a) Scientists understanding of the receptor for systemin is different this year compared to last year. What information changed their understanding?

**Ans.** This year it was discovered that signaling responses to systemin were neither increased in BRI1-overexpressing cells nor decreased in BRI1-silenced cells as compared to control cells. These results suggest the BRI1 can serve as a system-binding protein, but cannot transduce that signal into the cell as a true receptor would do.

(b) Regarding systemic acquired resistance (SAR), why is it called "systemic" and why is it called "acquired" (i.e., what event allows plants to acquire the resistance)?

**Ans.** SAR is called "systemic" because the SA induced at the original site of infection is transmitted as methylated SA to the rest of the plant, where it induces SAR through the vascular system. It is called "acquired" because this response is only acquired by the plant after pathogen attack.

(c) What is SAMT and what is its role in SAR?

**Ans.** SAMT is salicylic acid methyl transferase which esterifies SA with a methyl group. After pathogen attack, methylated SA is the preferred form that is transported to other parts of the plant, where it is de-methylated (converted back to SA) and induces SAR.

6. (a) State two genes turned on during pathogenesis, and indicate their value in defending plants against pathogens.

**Ans.** Glucanases weaken bacterial walls; proteinase inhibitors block ability of pathogen to gain nutrition from plant proteins; chitinase weakens fungal walls; peroxidase reinforces plant walls to make them more difficult to penetrate; defensin inhibits growth of bacteria and fungi.

(b) Describe evidence that the effects of SA are influenced by another hormone.

**Ans.** SA and ethylene synergistically induce an increased accumulation of PRI1 transcripts. To turn on this response a higher concentration of SA is needed when it is used alone compared to when it is combined with ethylene.

7. (a) Activated Gα is initially bound to a protein and to a non-protein compound. What are these 2 chemicals?

**Ans.** Initially, the protein bound would be an activated receptor; later activated Gα would bind to PLC. The non-protein compound would be GTP.
(b) When G-protein-linked receptors are activated, this often leads to a decrease in a certain compound present in membranes. What is this compound, and how does receptor activation lead to its decrease?

Ans.: The membrane compound that decreases is PIP2. Receptor activation activates Ga, which activates PLC, the enzyme that converts PIP2 to IP3 and DAG.

(c) What enzyme activity inactivates Ga and brings it back together with Gb and Gγ?

Ans.: GTPase

8. (a) Describe the steps by which a single hormone molecule outside a cell can lead to the production of a million signaling molecules inside a cell within 10 seconds. Your answer should include the phrase "rate of conversion of substrate to product" and should specify what increase in signaling molecules occurs at each step.

Ans.: Each hormone-activated G-protein-linked receptor could activate at least 100 G-proteins per second, and each of these could activate a PLC whose rate of conversion of substrate (PIP2) to produce is so fast, it could generate at least 100,000 IP3 and 100,000 DAG molecules per second. Thus, in less than 10 seconds, the binding of one hormone molecule could lead to the production of over a million signaling molecules in a cell.

(b) Some G-protein linked signal transduction chains involve the participation of a receptor that functions on an organelle inside the cell. What is one organelle that contains this receptor, what activates the receptor, and what change takes place in the cell after this receptor is activated?

Ans.: The ion-channel linked receptor for IP3 can be found on the ER or on the vacuolar membrane; IP3 activates it, causing the channel to open and allow calcium to flow out into the cytoplasm, thus increasing the [Ca²⁺]cyt and activating calcium-binding proteins there.

9. (a) What function of calmodulin links it to the fact that unstimulated cells typically have low [Ca²⁺]cyt? Explain your answer.

Ans.: Calcium-activated calmodulin can activate calcium pumps that pump calcium out of the cytoplasm thus lowering the [Ca²⁺]cyt.

(b) What does the acronym “CDPK” mean, and what is the relationship of the CDPK protein to calmodulin?

Ans.: Calcium-dependent protein kinase, or calmodulin-domain protein kinase. The structure of the CDPK protein has a region that is very similar to calmodulin.

(c) In measuring the effects of a stimulus on [Ca²⁺]cyt, why would it be useful to record how often the [Ca²⁺]cyt changed during the first minutes after the stimulus was given?

Ans.: The frequency of calcium spikes (peaks followed by troughs) induced by different stimuli can be different and these differences can lead to different downstream signaling steps, thus helping to confer more specificity to calcium signaling.