3

Immune Responses and Their Potential Role in Insect Outbreaks

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3.1 Introduction

In this chapter, we focus on the insect immune response as a potential mechanism that may facilitate pest herbivores' escape from regulation by natural enemies, allowing them to reach outbreak levels. We start by providing some background on the insect immune response and how its components have been experimentally measured. We go on to discuss how the immune response varies, why it is relevant to understanding the potential mechanisms or conditions associated with outbreaks, and investigate some traits of outbreak species that may be associated with an increased immune response. Although many studies have intensely examined factors that influence the insect immune response, to date only a handful of studies have examined the potential role of immunocompetence in outbreaks (Kapari *et al*. 2006, Klemola *et al*. 2007a, Ruuhola *et al*. 2007, Yang *et al*. 2007, Klemola *et al*. 2008, Yang *et al*. 2008). For this reason, we draw on the studies available and present several potential hypotheses (Table 3.1) to stimulate further research. Our goal is to show that changes in the strength of insects' immune responses may affect the success of natural enemies (parasites, parasitoids, and pathogens) in the host population, which in turn may destabilize insect–enemy dynamics, leading to outbreaks.

The role of parasites, parasitoids, and pathogens in regulating insect populations has been researched extensively. One clear hypothesis that has emerged from the outbreak literature is that outbreaks result from insect populations escaping the control of their natural enemies (Morris 1963, Berryman 1996, Klemola *et al*. 2010). Research to explain the mechanisms behind the escape from natural enemies include those that are behavioral (Gross 1993, Gentry and Dyer 2002, Smilanich *et al*. 2011), morphological (Gentry and Dyer 2002, Barbosa

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Table 3.1 Summary of hypotheses and evidence for how insect immune responses interact with outbreak potential.

Hypotheses	*Immunity measure*	*Hypothesis support*	*Comments*	*Species*	*References*
Density-dependent prophylaxis – hosts living at high densities suffer a greater risk of disease and therefore invest more in immunity.	M	–	Larval mortality from NPV declined as density increased.	*Mythimna separata**	Kunimi and Yamada 1990
	M	+	Resistance to granulosis virus increased as density increased.	*Mythimna separata**	Kunimi and Yamada 1990
	M	–	Larvae were most susceptible to infection at high densities.	*Mamestra brassicae*	Goulson and Cory 1995
	M	+	Larval transmission of viruses declined as density increased.	*Lymantria dispar**	D'Amico *et al*. 1996
	M	+	Larger egg masses had higher resistance to NPV.	*Malacosoma californicum p.**	Rothman and Myers 1996
	HM, M, PO	+	Higher densities were more resistant to fungus.	*Tenebrio moliator*	Barnes and Siva-Jothy 2000
	EN, PO	+	Immunity measures increased with increasing gregariousness.	*Spodoptera exempta**	Wilson *et al*. 2001
	AB, EN, HM, PO	+	Increased pathogen resistance under crowded conditions.	*Shistocera gregaria**	Wilson *et al*. 2002
	EN, HM, PO	–	Gregarious species had lower PO and HM.	Various Lepidoptera	Wilson *et al*. 2003
	EN, P	–	No difference in EN of moths from different sites.	*Epirrita autumnata**	Klemola *et al*. 2006

	M	+	Disease resistance increased as larval density decreased.	*Lymantria dispar**	Reilly and Hajek 2008
	M	–	Resistance to disease was not related to size of egg mass.	*Malacosoma californicum p.**	Cory and Myers 2009
Melanin – Outbreak species have higher cuticular melanin which is positively correlated with immune response.	M	+	Melanic morphs five times more resistant to fungus.	*Mythimna separata**	Mitsui and Kunimi 1988
	M	+	Melanic morphs two times more resistant to NPV.	*Mythimna separata**	Kunimi and Yamada 1990
	M	–	Melanic morphs were more susceptible to NPV.	*Mamestra brassicae*	Goulson and Cory 1995
	PO	+	Melanic morphs four times more resistant to NPV.	*Spodoptera exempta*	Reeson *et al*. 1998
	HM, M, PO	+	Darker morphs have stronger immune defense.	*Tenebrio moliator*	Barnes and Siva-Jothy 2000
	EN, HM	+	Positive association between size of wingspot and immunity.	*Calopteryx splemdens*	Rantala *et al*. 2000
	EN, PO	+	Melanic larvae more resistant to fungal disease.	*Spodoptera exempta**	Wilson *et al*. 2001
	EN	–	Darker individuals showed lower encapsulation and melanization.	*Hemideina maori*	Robb *et al*. 2003
	EN	+	Implanted pupae had darker melanization on wing as adults.	*Pieris brassicae*	Freitak *et al*. 2005
	M, P	–	Parasitism was positively associated with melanism.	*Operophtera brumata**	Hagen *et al*. 2006

Table 3.1 (*Cont'd*).

Hypotheses	*Immunity measure*	*Hypothesis support*	*Comments*	*Species*	*References*
	P	+	Higher parasitism in green larvae versus brown larvae.	*Macrolepidoptera*	Barbosa and Caldas 2007
	AB, PO	+	Causative relationship between melanism and immune response.	*Spodoptera littoralis**	Cotter *et al*. 2008
	AB, PO	+	High-protein diet increased melanization in cuticle and AB.	*Spodoptera littoralis**	Lee *et al*. 2008
	EN	+	Melanic morphs have stronger EN than pale morphs.	*Lymantria monacha*	Mikkola and Rantala 2010
Thermal effects – Continued increases in yearly temperature averages will lead to fluctuations in normal outbreak periodicity, due to altered immunity.	EN	+	Encapsulation of parasitoid highest during warmer months.	*Protopulvinaria pyriformis*	Blumberg 1991
	M	0	Virulence of fungus is strongly affected by temperature.	*Zonocerus variegatus*	Thomas and Jenkins 1997
	M	–	Melanization of silica beads decreased with increasing temperature.	*Anopheles gambiae*	Suwanchaichinda and Paskewitz 1998
	EN	+	Encapsulation of selected lines increased with temperature.	*Drosophila melanogaster*	Fellowes *et al*. 1999
	HM	+	Phagocytosis by hemocytes enhanced with increased temperature.	*Locusta migratoria*	Ouedraogo *et al*. 2002

	M	+	High temperature reduced susceptibility to parasitism.	*Acyrthosiphon pisum*	Blanford *et al*. 2003
	AB, AF	+	Heat-shocked larvae had enhanced immune response.	*Galleria mellonella*	Wojda and Jakubowicz 2007
Host plant – Species tend to outbreak on host plants that provide food quality conditions that are favorable for growth and immunity.	EN	+	Host plant on which larvae grew fastest also had highest EN.	*Pieris rapae*	Benrey and Denno 1997
	EN, PO	0	Nutritional conditions affected PO, but not EN of males.	*Tenebrio moliator*	Rantala *et al*. 2003
	EN	+	Larvae encapsulate on host plant with highest antioxidants.	*Parasemia plantaginis*	Ojala *et al*. 2005
	AB, EN, HM. PO	+	High protein content enhances NPV resistance and immunity.	*Spodoptera littoralis**	Lee *et al*. 2006
	EN	–	Hydolysable tannins have negative effects on EN.	*Epirrita autumnata**	Haviola *et al*. 2007
	EN, PO	–	Encapsulation was higher on low-quality foliage.	*Epirrita autumnata**	Klemola *et al*. 2007b
	EN, P	–	Larvae fed high- and low-quality diets did not differ in parasitism.	*Epirrita autumnata**	Klemola *et al*. 2008
	PO, AB	+	Larvae on high protein diet had higher AB activity.	*Spodoptera littoralis**	Lee *et al*. 2008
	AB, HM, M, PO	+	Increasing protein: carb diet increased resistance to infection.	*Spodoptera exempta**	Povey *et al*. 2009

Table 3.1 (*Cont'd*).

Hypotheses	*Immunity measure*	*Hypothesis support*	*Comments*	*Species*	*References*
	EN	+	Iridoid glycosides have negative effects on EN.	*Junonia coenia*	Smilanich *et al*. 2009a
	EN	+	Differential effects of amides on generalist versus specialists.	*Eois nympha, S. frugiperda*	Richards *et al*. 2010
	EN	+	EN increased with increasing host plant quality.	*Manduca sexta*	Diamond and Kingsolver 2011
	EN	–	Encapsulation not associated with resistance to pathogen.	*Orgyia antiqua**	Sandre *et al*. 2011
Key natural enemies – Certain natural enemies can circumvent the immune response. Outbreaks are predicted to occur when these enemies are less abundant.	AB, EN, PO	+	Parasitoid benefits from melanization of respiratory tube.	*Teleogryllus oceanicus*	Bailey and Zuk 2008
	EN, PO	+	Parasitoid "hides" from host immune response.	*Trichoplusia ni*	Caron *et al*. 2008
	PO	–	Polydnavirus inhibits phenoloxidase cascade.	*Manduca sexta*	Beck and Strand 2007
	EN	+	Polydnavirus inhibits phenoloxidase cascade.	*Manduca sexta*	Lu *et al*. 2010
	HM	–	Polydnavirus disrupts hemocyte function.	*Pseudoplusia includens*	Strand *et al*. 2006

Transgenerational effects and natural selection – Periods of high parasitism may be followed by outbreaks due to either maternal effects or natural selection.	—	+	Parental bacteria challenge increases resistance in progeny.	*Daphnia magna*	Little *et al*. 2003
	HM	+	Higher HM in populations from different potato fields.	*Leptinotarsa decemlineata*	Ots *et al*. 2005
	AB, PO, M	+	Parental immune challenge enhanced offspring immunity.	*Tenebrio molitor*	Moret 2006
	—	+	Natural selection for host resistance effects insect outbreaks.	*Lymantria dispar**	Elderd *et al*. 2008
	M	+	Transgenerational effect of crowding on pathogen resistance.	*Schistocera gregaria**	Miller *et al*. 2009
	PO, AB	+	Progeny of mothers exposed to bacteria exhibited priming.	*Trichoplusia ni**	Freitak *et al*. 2009
	AB, PO	+	Mothers and fathers can transfer resistance to offspring.	*Tribolium castaneum*	Roth *et al*. 2010

Abbreviations: EN, encapsulation/melanization; HM, hemocytes; PO, phenoloxidase; AB, lysozyme activity/ antibacterial activity; AF, antifungal activity; M, mortality (or survival); P, Parasitism, NPV, nuclear polyhedrosis virus.
* Denotes outbreak or pest species.

and Caldas 2007), chemical (Dyer 1995, Gentry and Dyer 2002, Nishida 2002), and physiological (Carton *et al*. 2008, Smilanich *et al*. 2009a). While each of these mechanisms is an important adaptation for escape from natural enemies, immunity is a critical escape mechanism that has received little attention (Godfray 1994, Smilanich *et al*. 2009b).

A low incidence of parasitism has been found to be characteristic of growing defoliator and other outbreak populations (Berryman *et al*. 2002). Indeed, the low incidence of parasitism in populations reaching outbreak status has often been attributed to the inability of parasitoids to "catch up" with the population growth of the outbreak population. Nonetheless, this conclusion has not accounted for the disparity between realized parasitism and actual parasitism. Parasitism rates are often assessed as the number of parasitoids reared from infected hosts (realized parasitism). This assumes that parasitism rates are singularly dependent on the number of parasitoids and/or the incidence of parasitism, and does not assess those insect hosts that have successfully defended themselves against parasitism (Kapari *et al*. 2006).

In long-term studies monitoring larval density and parasitism of the autumnal moth, *Epirrita autumnata* Borkhausen (Lepidoptera: Geometridae), on birch in Fennoscandia, *E. autumnata* was found to have delayed density-dependent variation in larval parasitism rates ranging from 0% to 100% (Ruohomäki 1994, Tanhuanpää *et al*. 2002). The abundance of some parasitoid species (surveyed from trapping of adults) around the time of outbreaks was found to decline to almost zero within two years after outbreaks (Nuorteva 1971). This suggests that the larval immune response to parasitism during periods of high larval density may play a role in decreasing the number of parasitoids post outbreak. Furthermore, Klemola *et al*. (2008, 2010) showed decreases in *E. autumnata* density with increases in parasitism and vice versa. Variations in herbivore–pathogen or herbivore–parasitoid interactions may be due, in part, to the susceptibility of the host herbivore to disease or parasitism. For example, the extent of oak defoliation by the gypsy moth, *Lymantria dispar* Linnaeus (Lepidoptera: Lymantriidae), was correlated with increased survival when exposed to nuclear polyhedrosis virus (NPV) (Hunter and Schultz 1993). Hunter and Schultz (1993) suggest that *L. dispar* was inducing changes in the leaf quality of its host plant in order to increase gallotannin concentrations and inhibit NPV. Another possibility in their scenario is that declining leaf quality due to defoliation may positively impact the immune responses of *L. dispar* against NPV.

3.2 The insect immune response

The immune response is composed of a humoral and cellular response that works in concert to defend against parasitoids, parasites, and pathogens (reviewed by Carton *et al*. 2008, Strand 2008, Beckage 2008). The cellular response is directed by specialized hemocytes. In general, when a special type of hemocytes called "granular cells" contacts a foreign target, they lyse or degranulate, releasing material that promotes attachment of plasmatocytes. Multiple layers of plasmatocytes then form a capsule around the object, which is then melanized by the

humoral response. A suite of proteins, including regulatory proteins, hemocyte response modulators, and melanization enzymes, are also involved in immunity, making it a complex and resource-costly process (Schmid-Hempel and Ebert 2003). The humoral response aids in the production of non-self-recognition proteins, enzymatic activity leading to melanization and clotting, and antimicrobial peptides. The combination of asphyxiation from encapsulation and the cytotoxic products produced during melanization is thought to contribute to killing the parasite (Strand 2008).

Experiments seeking to understand the variation in the immune response use a variety of methods. A common method for measuring encapsulation and melanization is injection or insertion of a synthetic object into the insect's hemocoel (Lavine and Beckage 1996, Klemola *et al*. 2007b). The immune response is quantified by measuring the color change and/or cell thickness around the object (Beckage 2008). Other measurements rely on quantifying the protein activity of the humoral response by determining the concentration of the enzyme, phenoloxidase (PO), which catalyzes the melanization cascade, or quantifying the activity of antibacterial lysozyme activity (Adamo 2004a). Still other measures include hemocyte counts (Ibrahim and Kim 2006), hemolymph protein concentration (Adamo 2004a), and gene expression (Freitak *et al*. 2009). Studies measuring the response of multiple immune parameters show that they do not always respond in the same way (Adamo 2004b) and can exhibit trade-offs (i.e., antibacterial efficiency trades off with PO activity) (Cotter *et al*. 2004b). For example, not only will a bacterial infection induce a specific component of the immune response that is different from a parasitoid infection, but also the response will be specific to the type of invading bacteria (Riddell *et al*. 2009). In relation to insect outbreaks, losing natural enemies that are capable of escaping the immune response may result in an outbreak. This idea will be further developed in the hypothesis section of this chapter.

3.3 Sources of variation in immune response associated with outbreaks

3.3.1 Host plants

Host plant diet is a major source of variation in the immune response (Smilanich *et al*. 2009a, Diamond and Kingsolver 2011). Since most aspects of herbivore outbreaks involve interactions with the host plant, it is reasonable to hypothesize that the immune response will play an integral role, from start to finish, in insect outbreak dynamics. The effects of host plants on immune responses can depend on plant chemistry, herbivore health, both herbivore and plant genotype, and the specific immune parameter being measured (e.g., PO activity, encapsulation of inert object, and lysozyme activity). Certain plant secondary metabolites can alter the effectiveness of immune responses. For example, ingestion of diets containing carotenoids enhances immune function due to their free radical–scavenging properties (de Roode *et al*. 2008, Babin *et al*. 2010). In contrast, ingestion of other secondary metabolites can negatively affect the immune response (Haviola

et al. 2007, Smilanich *et al*. 2009a). Macronutrients can also affect the immune response. Protein and carbohydrates not only boost immune parameters (Lee *et al*. 2008, Srygley *et al*. 2009, Cotter *et al*. 2011), as does plant and diet quality (Yang *et al*. 2008, Bukovinszky *et al*. 2009, Diamond and Kingsolver 2011) but also are preferred by immune-challenged herbivores (Lee *et al*. 2006, Povey *et al*. 2009). In most cases, high plant quality (i.e., low secondary metabolite concentration and high nitrogen content) translates to increased immunity (but see Klemola *et al*. 2007b).

3.3.2 Heritability

Different immune responses have been shown to be heritable, indicating that if immune responses play a role in outbreak formation or decline, there is a genetic component to the potential for a population or species to outbreak (Kraaijeveld and Godfray 1997, Schmid-Hempel and Ebert 2003, Carton *et al*. 2005). Early work investigating the genetic basis of immune defense mostly focused on *Drosophila melanogaster*, and has since expanded considerably to include many other invertebrate species (Cotter and Wilson 2002, Ojala *et al*. 2005, Moret 2006, Rantala and Roff 2006, 2007, Freitak *et al*. 2009). A significant heritability for encapsulation rate was found in *E. autumnata*, feeding on high- and low-quality host plants (Klemola *et al*. 2007b). Knowing the heritability and genetic basis of the immune response helps to understand and illuminate insect outbreaks in an evolutionary context. Later in the chapter we propose and discuss the hypothesis that the heritability of the immune response may play a prominent role in outbreak periodicity.

3.4 Traits or conditions associated with outbreak species

Only a small fraction of insect species reach outbreak proportions (Mattson and Addy 1975, Hunter 1995). Most species maintain a relatively low, stable population size and do not become noticeable defoliators (Mason 1987). Although there have been numerous studies trying to find traits or factors to explain why some species outbreak, we are still left with some rather unsatisfying explanations that in some way contribute to population regulation, from weather and host plant chemistry, to life history traits and natural enemies. However, it remains unclear why some species in the same habitat, under the same environmental conditions, are outbreak species and others are not. Furthermore, the questions of why some populations of species outbreak in certain geographical areas, but not others, are still the subject of much debate (Ruohomäki *et al*. 1997, Bjornstad *et al*. 2010).

Several studies have used a comparative approach between outbreak and non-outbreak species to examine important characteristics that set them apart (Mason 1987, Wallner 1987, Hunter 1995, Cappuccino *et al*. 1995, Price 2003). Hunter (1991) examined the Canadian Forest Insect Survey (CFIS) data supplemented with other sources to compare life history traits of outbreak and non-outbreak species of macrolepidoptera. The findings that are most relevant to our focus on

outbreaks and immune response include gregarious feeding behavior (Larsson *et al*. 1993, Cappuccino *et al*. 1995), overwintering as eggs in clusters or masses, and a wide diet breadth (a mean of 20 plant genera) (Hunter 1991). Although some advantages of gregarious behavior and laying eggs in clusters have been proposed (reviewed in Hunter 1991), we suggest that increased density of herbivores, which may be a result of egg clustering and gregarious behavior, may additionally lead to an increased immune defense against natural enemies. As discussed above, host plant attributes can alter the insect immune response; thus, polyphagy may be a trait that allows for adaptive responses in some outbreak species. The importance of these traits will be explored in the next section, where we propose several hypotheses for how the immune response plays an integral role in insect outbreaks (Table 3.1).

3.5 Hypotheses on insect outbreaks and the immune response

Hypothesis 1: Density-dependent prophylaxis (Wilson and Cotter 2009). This hypothesis proposes that hosts living at high densities suffer a greater risk of disease and therefore invest more in immunity.

Insects across a broad range of taxa are able to assess conspecific density, presumably as an indication of a potentially deteriorating quality or quantity of food, or exposure to pathogens or parasites (Carroll and Dingle 1996, Wilson and Cotter 2008). This is most often associated with insects that are able to migrate when conditions deteriorate (Denno *et al*. 1991, Carroll and Dingle 1996), or are capable of switching from a gregarious to solitary phase (Wilson and Cotter 2008). Given that not all insects are able to migrate when density increases (especially those in the larval stage or with limited mobility), such species may risk increased exposure to pathogens, parasites, and other natural enemies. With increasing density, the risk of transmission of disease increases due to the higher probability of interaction with potentially infected conspecifics (Steinhaus 1958, McCallum *et al*. 2001). Species that can modify their level of disease resistance to match potential risk of exposure would minimize the costly investment in disease resistance (Kraaijeveld and Godfray 1997, Wilson and Reeson 1998. Moret and Schmid-Hempel 2000). Many of the insects that exhibit density-dependent prophylaxis (DDP) are outbreak or pest species, suggesting that gregarious outbreak species may be more likely to allocate resources to immune defense as densities increase.

Resistance against parasites or pathogens may be costly to maintain and express (Sheldon and Verhulst 1996); therefore, a trade-off may exist between immune function and other traits. For example, Kraaijeveld and Godfray (1997) found a trade-off between competitive ability and immune function in *Drosophila* males. Natural selection should thus favor insects that are able to assess larval density and allocate more resources as needed for resistance against natural enemies (Goulson and Cory 1995, Wilson and Reeson 1998). This phenotypically plastic response of allocation of resources to defense has been demonstrated in numerous

insect species (Wilson *et al*. 2001, Cotter *et al*. 2004a, Ruiz-Gonzàlez *et al*. 2009). In a comparative analysis using 15 studies of temperate Lepidoptera, Hochberg (1991) found a positive correlation between gregarious feeding behavior and age-related resistance to viral infection.

Several studies indicate that there may be a connection between DDP and species that have been known to be outbreak pests (D'Amico *et al*. 1996, Rothman and Myers 1996, Wilson and Reeson 1998) (Table 3.1). For example, egg masses of the northern tent caterpillar, *Malacosoma californicum pluviale* Dyar (Lepidoptera: Lasiocampidae), range in size from 130 to 375 eggs per mass. Rothman and Myers (1996) orally exposed caterpillar siblings from a range of egg masses of different sizes to NPV in their second larval instar. They found that individuals that came from larger egg masses had significantly higher resistance to NPV than those from smaller egg masses. Although these differences associated with larval density may be attributed to resource availability, genetic differences, or maternal effects, density appears to play a role in intensity of resistance to viral infection across studies (Table 3.1).

The majority of DDP research indicates that there is a positive association between density and immune response (Wilson & Cotter 2009). There are still many aspects of DDP that warrant further investigation, such as how insects assess density, the trade-offs associated with increasing immune response, the specificity of immune responses, and the mechanism behind allocating resources for immune defense. Although several levels of density have been tested experimentally, whether there is a critical density threshold that facilitates an increase in immune response remains unclear. A large number of the studies examining DDP were conducted in the laboratory (Kunimi and Yamda 1990, Barnes and Siva-Jothy 2000, Cotter *et al*. 2004a). These studies may not allow for the most biologically relevant assessment of immune defense. Field studies on outbreak species, such as that of Klemola *et al*. (2007a) which assessed the immune response of *E. autumnata*, allowed for direct assessments of immune response at different densities. Examining density-dependent resistance under more natural conditions will allow us to gain a better understanding of how immune responses influence the population dynamics of outbreak species.

Hypothesis 2: Melanism and disease resistance. Outbreak species have higher cuticular melanin which is positively correlated with immune response

Phase polymorphism, a phenomenon closely associated with DDP, occurs when species change to darker, more melanized forms under high-density conditions (Wilson and Cotter 2009). Changes in color form are often correlated with other traits such as increased activity, respiration rate (Shibizaki and Ito 1969), desiccation resistance (Parkash *et al*. 2008), thermotolerance (Parkash *et al*. 2010), or flight activity (Parker and Gatehouse 1985). Phase polymorphism has been shown in a diverse array of taxa, including many lepidopteran agricultural pests or outbreak species (reviewed by Goulson and Cory 1995). It has been suggested that melanism may be a potential indicator of high levels of immunocompetence in insects (Majerus 1998, Barnes and Siva-Jothy 2000, Wilson *et al*. 2001). The

mechanism behind the association of melanism and immune defense remains unclear, although some studies have suggested possible mechanisms.

Cuticular melanin and the melanin involved in the immune response are sometimes correlated, thus successful outbreak species are more likely to have stages with increased melanin (Mikkola and Rantala 2010). Melanin is a product of the phenoloxidase (PO) cascade and is found in the cuticle, hemolymph, and midgut (Cotter *et al*. 2008). It plays a critical role in immune defense during recognition of foreign objects and encapsulation of large invaders (Wilson *et al*. 2001, Cotter *et al*. 2004b). It also improves the cuticle's ability to function as a physical barrier to the penetration of fungus, parasites, and pathogens (St. Leger *et al*. 1988, Hajek and St. Leger 1994, Wilson *et al*. 2001). An association between cuticular melanin and the insect immune defense against pathogens and parasites has been demonstrated in numerous species (Hung and Boucias 1992, Beckage and Kanost 1993, Wilson *et al*. 2001, Cotter *et al*. 2004b). Furthermore, melanin is toxic to microorganisms and has potential antimicrobial activity (Ourth and Renis 1993). Melanin is known to bind to a range of proteins and act as an inhibitor to many of the lytic enzymes produced by microorganisms (Bull 1970, Doering *et al*. 1999). The PO cascade involves a suite of enzymes that oxidize tyrosine to quinones and their polymerization product melanin (Nappi and Vass 1993). These same enzymes are also involved in cellular encapsulation, humoral encapsulation, and nodule formation (Hung and Boucias 1992, Beckage and Kanost 1993).

Although many of the studies mentioned showed increased melanism under crowded conditions, it is important to note that melanism is not just a by-product of increased density. Controlling for the effect of larval density, several studies have examined the association of melanism with disease resistance. At least four studies demonstrated that more melanic individuals had higher resistance against disease (NPV) or entomopathogenic fungi (Kunimi and Yamada 1990, Reeson *et al*. 1998, Barnes and Siva Jothy 2000). One similar study did not support these findings; Goulson and Cory (1995) found that melanic *Mamestra brassicae* Linnaeus (Lepidoptera: Noctuidae) larvae were less resistant to NPV compared to their less melanic conspecifics. The association of melanism and defense may be a highly specific interaction between herbivore host and natural enemy, such that in some cases the natural enemy may have evolved to overcome the herbivore host's defenses.

Conflicting data have been found on whether melanism is always associated with immune response, suggesting that it may be one of a number of factors that is important in immunocompetence. Using field data to quantify parasitoid attack rates in winter moth larvae, *Operophtera brumata* Linnaeus (Lepidoptera: Geometridae), Hagen *et al*. (2006) found that parasitoids were the greatest source of mortality (26%). However, cuticular melanin was positively associated with parasitoid attack (Hagen *et al*. 2006). Given that no research has definitively demonstrated that melanism is associated with increased immune response, there is a need for further research examining the role that melanin plays in immunocompetence, especially in outbreak species. The expression of melanism has been shown to be affected by a variety of environmental factors, including temperature and humidity (Goulson 1994), light (Faure 1943), and population density (Goulson and Cory 1995). There can be a high degree of phenotypic

variation between individuals and a significant effect of genetic family on melanism, indicating that melanism is heritable (Cotter *et al*. 2002, 2004a, 2008).

Hypothesis 3: Thermal effects on the immune response. Continued increases in yearly temperature averages will lead to fluctuations in normal outbreak periodicity, due to altered immunity.

This hypothesis centers on climate change and the projected increases in global mean temperatures. According to Mann *et al*. (2008), average temperature will increase by 4°C over the next 100 years. Undoubtedly, these temperature changes will impact insect populations and may disrupt regulation. The immune response will also be impacted by these changes since it is affected by temperature. However, the effects of temperature on the immune response are quite variable depending on the taxa, making a simple prediction between temperature and outbreaks impossible. Thomas and Blanford (2003) state that temperature can affect parasite virulence, host resistance, and host recovery. Thus, since multiple variables are affected by temperature, a complex relationship with many possible outcomes exists. For example, heightened temperature can enhance either natural enemy performance (Fellowes *et al*. 1999, Thomas and Blanford 2003) or the immune response (Fellowes *et al*. 1999, Wojda and Jakubowicz 2007), while in other cases it depresses natural enemy performance (Blanford *et al*. 2003, Thomas and Blanford 2003) and the immune response (Suwanchaichinda and Paskewitz 1998). Thus, the outcome of increased temperature will depend on the response of the enemy and the response of the host, especially where temperatures reach the boundary of normality based on their evolutionary history (Thomas and Blanford 2003). One pattern that has emerged from parasitoid–host data shows that increased variability in climate patterns will disrupt host tracking by parasitoids (Stireman *et al*. 2005), possibly leading to greater frequency in outbreaks. Whether this effect is due to increased immunity at higher temperatures is undetermined, yet holds implications for insect outbreaks. Greater immune defense at high temperatures could mean that parasitoids will not be as effective at controlling and maintaining insect populations.

With selection history in mind, dramatically altering temperature will at the very least lead to destabilization of normal insect–enemy interactions, which may lead to increased outbreak frequency (Parmesan 2006, Ims *et al*. 2008). Johnson *et al*. (2010) showed that outbreak periodicity shifts of the larch budmoth (*Zeiraphera diniana* Guenée), which exhibited regular 8–10-year outbreaks since 800 CE, is partially explained by increases in mean winter temperatures. Using a traveling wave model, they showed that increases in temperature at the optimal elevation for larch budmoth population growth lead to destabilization of outbreak periodicity (Johnson *et al*. 2010). In this case, the net effect of increased temperature is clear, but whether the immune response contributes to the destabilization is unclear.

Hypothesis 4: Host plant quality. Species tend to outbreak on host plants that provide food quality conditions that are favorable for growth and immunity. In most cases, these conditions will involve high nutrient quality and low levels of toxins.

As mentioned earlier, high-quality host plants enhance immune parameters, either directly (i.e., increased nitrogen for melanization precursors) or indirectly (i.e., increased body fat). However, the term "high-quality" should be defined in order to allow one to make a meaningful prediction. Here, we follow suit with most insect ecology literature and define quality based upon herbivore performance (e.g., development time, pupal mass, and fecundity) and nutrient profiles. In terms of nutrients, host plants that are high in nitrogen and water content are considered high-quality hosts since nitrogen is a limiting resource for insects, and insects risk desiccation from low water content (Scriber and Slansky 1981, Behmer 2009).

Most of the examples given earlier in the chapter deal with the macronutrient, protein, showing that high protein content enhances immunity (Lee *et al*. 2006, 2008, Povey *et al*. 2009, but see Cotter *et al*. 2011). Since the immune response relies heavily on enzymatic reactions that require amino acid precursors, it is fitting that immune-stressed herbivores would not only have enhanced immunity on high-protein diets but also prefer these diets to a nutritionally balanced diet. However, regardless of whether an herbivore is immune challenged, decades of research show that herbivores will regulate their nutrient intake, favoring a high protein-to-carbohydrate ratio (Behmer 2009). With this evidence, it is easy to speculate that a characteristic of outbreak species is the ability to achieve high protein content in their diets, either by consuming host plants with high protein: carbohydrate ratios and/or by superior nutrient regulation. For example, the gregarious tent caterpillar, *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae), is an outbreaking species that feeds preferentially on aspen and sugar maple. Research investigating the nutrient regulation of this forest pest shows that it does not regulate its diet to favor protein (Despland and Noseworthy 2006). Instead, its phenology is such that the beginning of its life cycle is in sync with new leaf flush on its host plants; this is a time when leaf nitrogen content will be high, which should favor a strong immune response.

Another reason why high-quality host plants may favor the occurrence of insect outbreaks is due to the documented trade-off between immunity and growth. Since the immune response is costly in terms of resources, it is predicted to trade off with other metabolic functions such as growth and fitness (Zuk and Stoehr 2002, Diamond and Kingsolver 2011). In other words, herbivores that are resource limited are not able to invest in both high growth and high immune capacity. For example, using path analysis, Diamond and Kingsolver (2011) found that host plant resource quality had a significant indirect positive effect on the encapsulation response via enhanced body condition (measured by growth rates) of *Manduca sexta* Linnaeus (Lepidoptera: Sphingidae) larvae. However, individuals with higher immune capacity exhibited slower growth. If outbreaking species are more likely to be found on high-quality host plants, then they may invest more in immune capacity than growth, which would favor escape from natural enemies. However, in some cases high-quality plants do not enhance immune capacity. Sandre *et al*. (2011) found that despite host plant–dependent resistance of *Orgyia antiqua* (Lepidoptera: Lymantriidae) caterpillars to the entomopathogenic fungus, *Metarhizium anisopliae* Sorokin, this resistance was not correlated with host plant quality or the encapsulation response, indicating a direct effect of host plant on the pathogen.

Not surprisingly, the effects of secondary chemistry on immune parameters are much more variable and dependent upon the action that the compound takes in the herbivore's body. For example, certain secondary metabolites, such as carotenoids, can increase the effectiveness of the immune response by ameliorating the autoreactivity of the melanization cascade (de Roode *et al*. 2008, Babin *et al*. 2010). However, ingestion of hydrolysable tannins and imides, and high concentrations of iridoid glycosides, reduced encapsulation and melanization (Haviola *et al*. 2007, Smilanich *et al*. 2009a, Richards *et al*. 2010), while still other secondary metabolites have no effect on the immune capacity (Smilanich *et al*. 2011). These differing results reflect the enormous diversity of secondary metabolites and the role of evolutionary history between herbivore and host plants. In short, overall host plant quality will most likely be a better predictor of immune capacity than specific secondary metabolites.

Hypothesis 5: The key natural enemy hypothesis. Even though the immune response is an effective defense, certain natural enemies can circumvent the immune response. Outbreaks are predicted to occur when these enemies are less abundant.

Since the immune response is one of the most effective defenses against parasitic enemies (Smilanich *et al*. 2009b, Godfray 1994), it is evolutionarily fitting that these enemies will have evolved counter-adaptations to cope with or suppress the immune response. Indeed, the best example of a counter-adaptation to the insect immune response is exhibited by hymenopteran parasitoids in the families Braconidae and Ichneumonidae (Webb and Strand 2005). Species in these two families harbor polydnaviruses, which have become integrated into the wasp's genome and are passed vertically through the germ line to offspring (Strand 2009). The virus replicates in the reproductive tract of the female wasp and is injected into the host during oviposition (Beckage 2008). Once inside the host's hemocoel, the virus infects immune-functioning cells, enzymes, and tissues such as hemocytes, phenoloxidase, and fat body, and thereby suppresses the immune response (Strand 2009). Another example of counter-adaptation to the immune response is found in certain species of tachinid flies. Bailey and Zuk (2008) found a positive correlation between the phenoloxidase activity of the field cricket, *Teleogryllus oceanicus* Le Guillou (Orthoptera: Gryllidae), and the melanization of the respiratory funnel in the attacking tachinid fly, *Ormia ochracea*. Since the respiratory funnel is the means by which many tachinid flies receive oxygen, a stronger funnel that is less likely to break is beneficial. In this way, these flies may be co-opting the immune response for their own benefit as the funnel is strengthened by the encapsulation process. Other species of tachinids have evolved a behavioral counter-adaptation to the immune response. The broad generalist tachinid, *Compsilura concinnata*, hides from the host immune response by developing between the peritrophic membrane and the midgut, where the immune response has limited access (Caron *et al*. 2008). Similarly, other tachinids reside in certain tissues, such as fat bodies, to avoid the immune response (Salt 1968).

In insect populations, these natural enemies that are capable of suppressing or circumventing the immune response may be key sources of mortality. When

populations of these key natural enemies are low, it may lead to outbreak situations. Moreover, insect species with the fewest key natural enemies will be the most likely to outbreak. Recent models by Bjørnstad *et al*. (2010) and Dwyer *et al*. (2004) demonstrate that population cycles of outbreaking insects are determined by a complement of natural enemies. In particular, Bjørnstad *et al*. (2010), show that the periodicity of outbreaks in *L. dispar* populations is governed by both generalist and specialist natural enemies. Generalist predators maintain the population up to a certain carrying capacity at which point an outbreak occurs. The outbreaking population is brought back to pre-outbreak size by a specialist pathogen that is capable of escaping the immune response. Similarly, Dwyer *et al*. (2004) use a host–pathogen–predator model to show that generalist predators maintain the population whereas specialist pathogens maintain the cycles. Although these data are not a perfect demonstration of our hypothesis, they support the idea that different types of natural enemies each play a role in outbreak cycles. Both examples hinge on the premise that the natural enemy is capable of escaping host defenses. In general, whether or not key natural enemies are avoiding or overcoming the immune response, and whether they are specialists or generalists, have yet to be tested.

Hypothesis 6: Transgenerational maternal effects and natural selection. Periods of high parasitism may be followed by outbreaks due to the following: (1) transgenerational maternal effects where individuals that survived parasitism attack have a heightened immune response and produce offspring that are immune primed, or (2) individuals surviving the attack are genetically selected for higher resistance, and progeny of these individuals will also have higher resistance via inheritance.

Data sets monitoring caterpillar density and numbers, and monitoring parasitism, support this hypothesis (Karban and Valpine 2010, Schott *et al*. 2010). These data sets show peak periods in natural enemy populations followed by peaks or outbreaks in insect populations. In addition, host–pathogen models predict this same trend where periods of high parasitism are followed by outbreaks (Elderd *et al*. 2008). Although the empirical data sets focus on caterpillars and parasitoids, the models are general for outbreaking species. An exception to this trend is seen in the data set with *E. autumnata* (Klemola *et al*. 2007a, 2008, 2010). This data set started in the 1970s and shows periods of high parasitism matching periods of low caterpillar density. In addition, Klemola *et al*. (2008) found no relationship between *E. autumnata* immunity and parasitism status, suggesting an alternative mechanism regulating population outbreaks for this caterpillar. Thus, our hypothesis may be most relevant for populations that rely heavily on the immune response to resist pathogens and parasites.

Transgenerational maternal effect

A spike in natural enemy population (pathogen, parasitoid) leads to many individuals in the population attacked and presented with an immune challenge. Epigenetic mechanisms may occur such that the progeny of immune-challenged and surviving parents are immune-primed against another attack. This may occur through mechanisms such as maternal effects, where the physiological result of the mother's

developmental environment is passed on to offspring. Other genetic mechanisms are also possible, such as chromatin marking, where the structure of the DNA molecule is altered by an environmental stimulus (Jablonka and Lamb 2010). Transgenerational priming of the immune response has been shown in insects, although the exact mechanism is unclear (Little *et al*. 2003, Moret 2006, Freitak *et al*. 2009, Roth et al. 2010). The best evidence to date is that of Freitak *et al*. (2009), in which Trichoplusia ni Hübner (Lepidoptera: Noctuidae) progeny whose mothers were exposed to dietary bacteria exhibited transgenerational priming of enzyme activity, protein expression, and transcript abundance of immune-functioning genes. While these results support maternal effects of the immune response, the offspring's response did not exactly mirror the mother's response. Studies have shown that the maternal effects are fleeting, and without the stimulus will not be maintained in the population (Jablonka and Lamb 2010). Thus, if natural enemy populations are not as high during the F1 generation, the maternal effect is not as prominent in the population, and the outbreak subsides in the F2 generation.

Natural selection

Periods of insect outbreak following periods of high parasitism may be due to evolutionary processes such as natural selection. Regardless of maternal effects, individuals that have strong immune responses will be more likely to survive an attack, and, if heritable, these genes will be passed to the next generation. Using both a model and experimental approach, Elderd *et al*. (2008) demonstrate that resistance to natural enemies plays a prime role in population fluxes of the L.dispar and that natural selection for resistance to these enemies drives population cycles. In this scenario, it is more difficult to explain the end of an outbreak if the progeny are strongly resistant; however, a wealth of additional factors, both physiological and ecological, can result in population crashes.

3.6 Conclusions

Outbreak species share traits and behaviors that implicate the immune response as having the potential for playing a significant role in insect outbreaks. Their gregarious behavior and egg clustering, which increase the density of conspecifics in a given area, may increase their likelihood of density-dependent prophylaxis and melanism (which in turn strengthens their resistance to natural enemies). Outbreak species are often polyphagous, allowing for the potential of differential host plant selection, sometimes resulting in the selection of diets which maximize immune defense when needed. As research on the insect immune response continues to accumulate, the rather coarse picture that we have drawn of the role of the immune response will be refined such that exact mechanisms of immunity on outbreaks can be defined and described. There are many avenues to explore on this topic, and the six hypotheses that we presented can be used as guidelines for future research (Table 3.1). These hypotheses are not mutually exclusive and most likely work in concert to influence outbreaks. Most of the examples pooled in this chapter come from research on forest insect pests, yet the hypotheses can likely be generalized to agricultural pests.

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