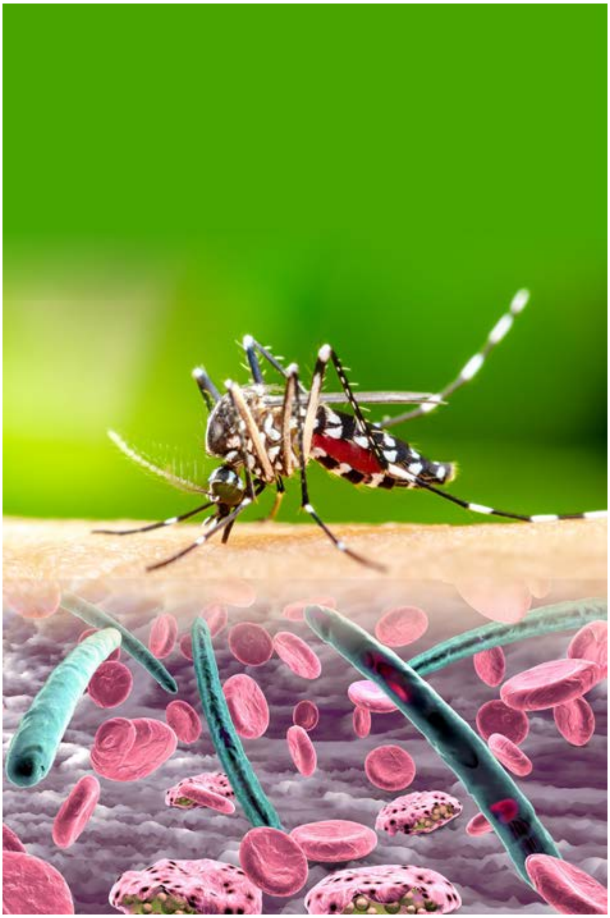
CHAPTER FIVE

Three scenarios in insect-borne diseases



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Insect-Borne Diseases in the 21st Century
https://doi.org/10.1016/B978-0-12-818706-7.00005-X

Insect-borne diseases affecting mankind

Reading in the books the history of each country, at any time we can find sequences of episodes about outbreaks due to the plague and other epidemics. Traces of parasitic diseases on humanity can be easily found in ancient Egyptian civilization, where pests and plague were considered as inherent and ineluctable parts of Creation:

> *"Who creates that on which the mosquito lives, worms and fleas likewise, who looks after the mice in their holes and keeps alive the beetles in every timber."*
>
> ***(From the Hymn to Amen-Re, c.1600 BC, after Jan Assmann Ägypten*—Theologie und Frömmigkeit einer frühen Hochkultur*, p. 73)***

Also in the Bible, the devastating effects of pests and plagues are clearly present, but the concept regarding the origin and the acceptance are very different from the Egyptian ones.

> *"I will send swarms of flies on you and your officials, on your people and into your houses. The houses of Egyptians will be full of flies, even the ground will be covered with them."*
>
> ***(Exodus 8: 21)***

Among Egypt's plagues in the Bible, at least four can be directly related to parasites and their vectors. The description of these episodes is full of a sentiment of collapsing certainty in the face of unchained nature's forces. There is a feeling of mankind's impotence in particular against insect-borne diseases until an endless darkness.

> *"Stretch out your hand toward the sky so that darkness will spread over Egypt—darkness that can be felt."*

A very impressive description of the plague's (or a similar disease) incumbency is contained in the description of the Four Horsemen of the Apocalypse in the last book of the New Testament of the Bible, the Book of Revelation by John of Patmos, at 6:1–8. "I looked and there before me was a pale horse! Its rider was named Death, and Hades was following close behind him." The identities of the four horsemen are then summed up as follows: "They were given power over a fourth of the earth to kill by the sword (war), famine, and plague and by the wild beasts of the earth."

Other references to the deep relationship between mankind's life and insect-borne diseases can be found everywhere, especially in poetry. In these texts, we can find desperation and horror about terrible pain, but also descriptions of how mankind can transfigure even terrible pests in beauty, conferring on the disease the respect due to a shining divinity. Homer in the *Iliad* refers to "the star they call Orion's Dog—brightest of all but a fatal sign emblazoned on the heavens, it brings such killing fever down on wretched men." We find again fever and Troy in Dante's *Inferno*: "Master Adam yawns and introduces them to Potiphar's wife [who falsely accused Joseph of raping her] and Sinon of the Greeks, who tricked the Trojans into taking the Trojan horse inside their city walls. Both are afflicted by a fever so fierce that it makes their skin smoke." But he also gives a precise description of the symptoms: "Like those who shake, feeling the quartana fever coming on—their nails already blue, so that shiver at the mere sight of shade—such was I then."

Nowadays, the impact of insect-borne diseases can be monitored and determined. In the period 2010–2013, 832,900 annual deaths were estimated for parasitic infection, including: malaria, 584,000; cryptosporidiosis, 100,000; amebiasis, 55,000; leishmaniasis, 51,600; schistosomiasis, 11,700; Chagas disease, 10,300; cysticercosis, 1200; and food-borne trematodiases, 7000. However, these numbers are only indicative, since most infections do not show symptoms. Also in this aspect, there are immense variations in many parts of the world. In any case, it is evident the impact of insect-borne diseases in indelibly passages in the story of the Anthropocene Age.

Lessons from the past

There is a sort of ancient memory transmitted through generations about dangers around us. Some people exhibit ancestral well-founded fears against some animals. Africans do not like snakes, whereas Europeans are horrified by rats, which are considered primarily responsible for diffusion of plague (McCormick, 2003). In human memory, the most devastating arthropod-borne disease is not malaria or dengue or Ebola, but the plague named the Black Death and in Europe the Black Death is still considered the plague par excellence (Carter and Mendis, 2002; Benedictow, 2004).

The plague is caused by the Gram-negative bacillus *Yersinia pestis*, only 2 μm long, which is a coccobacillus. The infection to humans takes a disease in three main forms: pneumonic, septicemic, and bubonic plague. The last one is the most evident and famous. Its transmission is in accordance with classic sequence of arthropod-borne disease: pathogen, vector, target. In this case, the vector is *Xenopsylla cheopis*, the Oriental flea, which feeds on an infected animal and ingurgitates the blood containing the parasite cells. Inside the intestine, *Y. pestis* starts to multiply and create a mass of cells, which appears as a dark mass in the gut. Several proteins then contribute to the maintenance of the bacteria in the flea's digestive tract, among them the hemin storage system. This is a mechanism to increase the infection: when the foregut of the flea is blocked by the *Y. pestis* biofilm complicating the digestion of the nutrient, the flea needs to eat again, and makes multiple attempts to feed. In this way, *Y. pestis* is regurgitated into the wound of another host, causing infection.

Y. pestis is deprived of any form of locomotion and unable to propagate by spores, but its method of transportation is very efficient. Initial acquisition of *Y. pestis* by the vector occurs even during feeding on an infected animal and *Yersinia* murine toxin (Ymt) plays its role. Ymt is highly toxic to rodents and was considered important to ensure reinfection of new hosts and for the survival of *Y. pestis* in fleas. Rodents are actually not highly affected by *Y. pestis* and other similar parasites, whereas rats remain the ideal hosts. Focusing on human targets, it is necessary to note the central role of the flea: our skin is usually a good barrier against infection, but the bite overcomes this barrier.

As with other epidemics, this plague is characterized by a turnover of expansion and contraction, long periods of silent inactivity followed by furious activity. Two in particular were described by direct reliable reporters, which depicted the facts and their astonishment at the entity of the phenomenon. The first one is located at the decline of Roman Empire in the 6th century CE, and the second in the 14th century. Histories of the Great Plagues have been recently rewritten thank to the analyses of buried victims' DNA. Michael McCormick, an archeologist and medieval historian at Harvard University, reported in a published paper that the year 536 was the worst one in history to be alive (McCormick, 2003). His consideration is based on the documents of that time and confirmed by the analysis of ice carrots collected from the top of Mont Rosa, in the Colle Gnifetti permanent glacier, evidencing the effects of a giant eruption. Similarly, in Antarctica and Greenland glaciers showed volcanic debris from 540, indicating a second eruption. The ice layers tell us of a blanket of fog and ash darkening the sun for years in Europe, the Middle East, and large parts of Asia, causing temperatures to plummet in the summer to between 1.6 and 2.5 degrees across these continents. Day and night in a perennial eclipse, the abnormally cold summer spurred the coldest decade in the past 2300 years. Production of food, with no bread, was affected for at least 5 years. This is the time necessary for the environmental *sudarium* to leave its definite mark on an afflicted and desperate humanity.

Once again, we entrust our possibilities to understand the disease's start and spreading, through the correlation with the sequence of the environmental events occurred in the considered period. In 535, in the volcanic archipelagos of Indonesia, including the ill-famed Krakatoa, a giant explosion occurred, followed by enormous eruptions, and generated a gigantic cloud of ash and lapillus. The cloud moved to the Occident, reaching China and later Europe. We have no direct historic reports of that time from Java, but this is considered a sign of the scale of the disaster. We can only imagine what kind of tsunami and devastation were generated. First, the Chinese historians wrote of a terrible thunder coming from the south and shaking the very ground. This was only the beginning of the catastrophe. Later, an immense cloud raised and covered the sun and the darkness reached Europe, where several historians of the time directly reported the experience. About 10–80 cubic km of incandescent material was projected into the sky, producing a cloud 20–150 m thick, which slowly covered most of the planet. Michael the Syrian reports that the sky became dark and the darkness lasted for 11 months, like an eclipse. The dendrochronology of fossil wood confirms this event. Krakatoa is far from Europe, but another contemporaneous eruption took place in Island. The environment was highly affected twice. According to the current opinions of scientists, the first consequence was a global decrease in temperature: 5–10 degrees in about 10–20 years. Suddenly, the next year (536) everything changed. A warming, caused by greenhouse gases and loss of ozone protection, burned the same places. Later, until 565 the temperatures were very low, but later again there was a hot period, with temperatures much higher than those we are experiencing now. To have an idea of how rapid the change was, between 1929, which had a freezing winter, and the heatwave of 2009, we had an average annual difference of half a degree. In Italy, the weather conditions between 540 and 590 looked completely unstable. In 590 abundant and continuous rains caused inundations, even in Rome as never before ("rivers devastated Flaminia route as ploughing, the banks of greatest bridges joined together, pressed by an inundation extremely virulent from woods," as observed by Cassiodorus). The account of Paulus Diaconus is impressive:

> *In hac diluvii effusione in tantum apud urbem Romam fluvius Tiberis excrevit, ut aquae eius super muros urbis influerent et maximas in ea regiones occuparent. Tunc per alveum eiusdem fluminis cum multa serpentium multitudine draco etiam mirae magnitudinis per urbem transiens usque ad mare discendit. Subsecuta statim est hanc inundationem gravissima pestilentia, quam inguinariam appellant. Quae tanta strage populum devastavit, ut de inaestimabili multitudine vix pauci remanerent. Primumque Pelagium papam, virum venerabilem, perculit et sine mora extinxit.*

> *In such greatness of water produced by the deluge, the river Tiber inundated Rome, so that the water overcame the town's walls and was able to occupy all the areas. Thus, from its riverbed a great number of snakes, together with an enormous dragon, crossed the city until they descended to the sea. Then, a very serious epidemic immediately followed the flooding, which was called* inguinariam *[bubonic]. Such great carnage devastated the people, so that those from the incalculable multitude, barely a few people survived. The Pope Pelagius, the venerable, faltered and without delay extinguished.*

In contrast, the following year saw a terrible drought, with no rain between January to September and a devastating invasion of locusts in the north-east regions of the peninsula. Temperature and moisture are the most important factors in a habitat, modeling presence of living organisms, and causing migration, usually in combination with biotic influences.

A new pope was immediately elected and he took the name of Gregorius I, later named as the Great. The importance of Gregorius was immediately clear to the population of Rome. Considering the catastrophic situation, the pope called the entire population of the town to participate in a historic procession. From seven parts of Rome, all people converged in Santa Maria Maggiore Cathedral, asking for God's help and compassion. From a medical point of view, an overcrowded square was not the best idea against the plague, but, according to the legend, the archangel Michael appeared to Pope Gregory I and the Roman population. Whether one believes this or not, the pestilence in Rome stopped, and the castle's name was changed in honor of the episode. Now, if you go to Rome, you may well visit St Angel Castle, which is topped by a bronze statue of an angel sheathing his sword, as a sign of the end of the plague. This is also where Tosca completed her tragic destiny in Puccini's opera.

However, Rome's pestilence was not the worst episode of that year. After the fall of the Roman Empire, the Pax Romana was followed by a period of conflicts and confusion. Wars are a tremendous well of inspiration for writers and a source of precious information for us about what was going on in ancient times. Thanks to *De Bello Gallico*, we know something about Celtic civilization, before its leveling by Romans and its integration into the Empire. Homer's *Iliad*, Thucydides' *History of the Peloponnesian War*, other reports from Hippocrates (Pappas et al., 2008), and even Tolstoy's *War and Peace* and several of Shakespeare's plays are fascinating accounts of wars full of information and descriptions of life at that time. During the military campaigns, besides the combating troops there is a plethora of writers, experts, and counselors of different types, which reported the facts and events with their own impressions and points of view, such as in the Second World War, when the conflict in Europe was fully documented by photographers and film makers.

Procopius of Caesarea was a historian living in the period of the great plague in Europe in the 6th century. He wrote in seven books, named the *History of the Wars* (or simply *Wars*), the events of that period, describing the conflicts which characterized that convulse years. In that period, wars were the result of the confrontation between the waves of Barbarians, coming from the North and the Orient, and the remaining part of the Roman Empire under the Emperor Justinian, whose explicit intent was the reunification of the Empire. During wars there are people moving to conquer and people moving to defend. The result is that incoming people can bring some diseases, but usually they are easy prey for pathogens. Contrary to a popular opinion, there are evidences that the mass migration of people moving from Africa to Europe is not bearing diseases, on the contrary these people are subjected to local strains of pathogens. At that time, ordinary people had to find refuge in the fortified towns, which thus became overcrowded and faced long sieges. It was necessary to store a great quantity of food, mainly as cereals; more grains meant more rats, and more rats meant more fleas, everywhere. Rats are very intelligent and well-adapted to live in our habitat. In our towns, there are more rats than humans, but these millions of rats are not visible and they are able to conceal themselves and come out under cover of darkness. We observe their presence through their excrement and the consequences of pathogenic activity related to them. According to reports, a virulent strain entered Rome from Egypt, probably derived from China and dormant for a long time, incubating in the central region of the Great Lakes. In Roman times, Egypt was called the granary of Rome and it supported for a long time the needs of imperial Rome. A boat full of grain was probably the transportation through which infected rats moved from the Nile to Gaza and later the European continent. In *The Persian War* (2–22) Procopius described carefully the beginning and the lent progression of the pestilence touching any village or island, until it arrived in Byzantium, the capital of Justinian's Empire. In a few weeks, the plague found its ideal habitat, where density of human settlement and lack of hygiene played vital roles for the spread of the disease. At that time, the town contained 500,000 citizens, with enormous naval traffic.

Once the parasites overcame the initial difficulties, the consequences were a series of devastating attacks. The first period accounted for 5000 deaths every day, rising to 12,000, until after 12 days the town had lost 10% of its population. The Justinian plague raged for 3 months causing an emergency in terms of managing the enormous number of corpses (Rosen, 2007). Cadavers were thrown out from the walls and amassed on the rocks below until their removal by the sea. Byzantium was a putrescent grave in the open air. Later, all of central Europe was affected, including Rome, as we have seen.

This is the story, but the interpretation of facts is different. In his book *The Secret History*, Procopius describes Justinian as like a devil and the source of any damage, accusing him of being responsible for the fall of the Roman Empire, but the plague was probably a fundamental cofactor. It continued for centuries until 700 CE and the victims are estimated at 40%–50% of the population. Finally, the plague receded, but lurked for centuries until in 1347 it hit Europe again.

In the mid-14th century, Europe again experienced a great plague, carried in the guts of fleas that rode on the backs of black rats from China. The port city of Caffa in Crimea on the Black Sea was thought to be the location of the infestation's origin (Fig. 5.1). Again, a merchant boat from Caffa returned to Italy, infecting Genoa, and the disease spread throughout Italy and later the Mediterranean Basin and all of Europe.

After eight centuries and many generations, the pestilence reappeared with the same virulence. The new outbreak, named the Black Death, is estimated to have killed 30%–60% of Europe's total population, affecting this

Fig. 5.1 Pestilence and other diseases were attributed to the actions of devils and considered to be a consequence of some sin or deviance.

time both towns and rural sites. It is considered the most devastating pandemic in human history, causing 75–200 million of deaths in Eurasia from 1347 to 1351. The horror of that period is still evident outside the cathedrals of central Europe, with the gargoyles threatening a dispersed and frightened humanity, and inside dominant pictures of triumphant Death armed with his terrible scythe (Fig. 5.2). Again, effects were cultural and social, changing the course of history, and again, most people considered the plague to be God's punishment. Few other than those in religious orders dared to nurse the sick. After just 4 years, two houses out of every three were totally empty and the astonished survivors scattered around without any hope in the future. Finally, the epidemic curve changed and the future became brighter. However, this tragedy launched an evolutionary transformation in Europe that deviated the course of history, causing social and cultural revolutions such as Lutheranism. People expected protection from the Church, and paid for expensive indulgences. The Church shockingly failed to influence human affairs and its authority was challenged by many, including Chaucer in his mocking *Canterbury Tales*. In a sense, the Black Death was the incubator of enclosure and of the Reformation. On October 31, 1517, Martin Luther nailed his 95 Theses to Wittenberg's cathedral doors, asserting that indulgences were not valid. The tripartite medieval division of society, the revision of that of our Arian precursors—those who fought (the nobility

Fig. 5.2 Death dominated without any possibility of interfering in its course. The personification of death is common in the imagery and art of that period.

Fig. 5.3 The masks used for centuries in the past as protection against the plague and now sold as souvenirs in the shops of Venice.

and knights), those who prayed (the churchmen), and those who labored (the peasants)—was revolutionized, never to be the same again (Fig. 5.3).

The scenario of the next episode is London, the new capital of Europe. The sequence of events is more or less the same, as well as the virulence. From the south, probably Netherlands, a boat carrying cotton arrived at the docks of London, which was the first area to be affected by the pestilence, now named Bubonic Plague. The date April 12, 1665 marks the first record of a victim, Rebecca Andrews, but the plague was already affecting thousands of very poor workers living in crowded and bad conditions, whereas those who could, including most doctors, lawyers, and merchants, fled the city; even the King and Parliament went to Oxford. Nobody knows how many of these people may have died from the plague. Between 1665 and 1666, 200,000 deaths are estimated, corresponding to one-quarter of the population, until the Great Fire of London destroyed much of the city, but also killed most of its rats and fleas. The Great Plague also hit other parts of Europe; in Amsterdam, the disease killed about 50,000 people. The plague killed 30%–60% of Europe's total population, reducing the world's population to 125–150 million (Benedictow, 2004). After such virulence, too many deaths, and immense devastation, what stopped such immense biological power? So far, no one has been able to furnish a convincing explanation. A.R. Appleby (1980) explained everything by the key role of the rat-flea-human pathway. When the rats acquired immunity, the plague suddenly disappeared. Others observed that when the plague spread over London during 1665, there was a higher prevalence of blood-sucking bugs in humans at the time of the Black Death than in recent times. On the other hand, the human-to-human transmission by way of these ectoparasites has been advanced as a route that could speed up the spread of plague: fewer humans, less infection.

We are living periods characterized by an alternance of violent outbreaks, under the attack of virulent parasites, and relative peace, when the pathogens are silent. In 1855, the third pandemic plague began in Yunnan province of China. As expected, Bubonic Plague spread from the Orient to other parts of inhabited continents. However, other acts must be expected. There are reports that more than 12 million people died, with 10 million people killed in India alone. According to the WHO, thousands of cases of the plague are still reported every year, although with proper treatment, the prognosis for victims is now much better, thanks to treatment with antibiotics. In 2000, the WHO's Report on Global Surveillance of Epidemic-prone Infectious Diseases stated:

> *"Plague has declined dramatically since the early part of the twentieth century, when outbreaks could cause tens of millions of deaths. This is due primarily to improvements in living standards and health services. However, a substantial number of countries continue to be affected by plague, case fatality rates remain high and antimicrobial resistance has begun. Therefore, continued vigilance is required, particularly in human populations living near natural plague foci. Plague foci are not fixed, and can change in response to shifts in factors such as climate, landscape, and rodent population migration. Natural foci of plague are situated in all continents except Australia, within a broad belt in tropical, subtropical and warmer temperate climates, between the parallels 55° N and 40° S."*

Finally, we are ready to use knowledge about the Great Plague as a classic model of insect-borne disease for some considerations.

A never-ending story

This is a never-ending story, every time different and still evolving. What is the current real impact of insect-borne diseases? What are the forces on the battlefield, and the alliances? Is it a global challenge or we are going to solve

Table 5.1 Most important diseases caused by infections due to microorganisms or viruses and their incidences in mortality.

Disease	Annual global cases (millions)	Annual global deaths (millions)	% Between death and cases
Malaria	600	3–8	0.005–0.013
Dengue	96	0.02	0.0002
Tuberculosis	8.6	1.300	0.151
Influenza	4	0.375	0.093
HIV	2.3	1.600	0.695

this eternal problem eventually? Although these are the key questions, replies are not easy to find.

Data reporting the current situation concerning insect-borne diseases are summarized in Table 5.1. They can be divided into two main groups on the basis of the etiological agent, which could be a virus or a bacterium. In any case, a specialized flying mosquito acts as an efficient vector, transferring the agent by bite into the host. Therefore, several aspects of the diseases are common, although important differences must be evidenced, as symptoms, diffusion, incidence, and mode of action, influencing also the strategy and method of control.

In 2006, the World Health Organization (WHO) declared the mosquito vectors for the transmission of diseases as "public enemy number one." The concern of the WHO was based on numbers indicating global impact and the need for new solutions. Insect-borne diseases are prevalent in more than 100 countries across the world, infecting more than 700 million people every year globally, with India alone accounting for 40 million and resulting in more than 1 million deaths each year. The main diseases transmitted by blood-feeding mosquitoes, according to the WHO, include dengue fever, dengue hemorrhagic fever, Japanese encephalitis, malaria, and filariasis. In particular, lymphatic filariasis has been reported to affect at least 120 million people in 73 countries, including Africa, India, Southeast Asia, and the Pacific Islands. In India, global filariasis constitutes around 40% of global filariasis. Japanese encephalitis accounts for an annual incidence of 30,000–50,000 with a mortality estimate of 10,000. These diseases are increasing in prevalence, particularly in tropical and subtropical zones, causing morbidity, mortality, economic loss, and social disruption.

However, the above data are not enough to evidence the impact of insect-borne diseases. As with any science, but in particular social sciences, data can be considered, or interpreted, in different ways, i.e., in total amount or in relative percentages, but usually the data are manipulated to be coherent with the idea to be supported. In addition, the interpretation can be affected by the intention of the writer or the reporter. In this way, if we consider the percentage of mortality of worldwide important infective diseases, as reported in Table 5.2, we find that there are more dangerous sicknesses than the diseases considered in this book. However, these data are also very partial considering that in many cases, most people at risk or infected do not become sick or are able to coexist with the disease.

What is the truth about the future of insect-borne diseases? The only possible consideration is that in this scenario, to control mosquitoes and mosquito-borne diseases, and decrease health and economic impacts worldwide, synthetic insecticide-based interventions are necessary, particularly in situations of epidemic outbreak and sudden increases of adult mosquitoes. However, the utilization of insecticides is going to change or disappear, in the next 20–30 years for reasons related mainly to the resistance phenomenons.

Table 5.2 Major vector-diffused diseases.

Disease	Vector	Pathogen	Impact and other data
Dengue, chikungunya fever, urban yellow fever	*Aedes aegypti*	Chikungunya virus	3.5 Million people at risk in 128 countries
Zika	*Aedes aegypti*	Chikungunya virus	About 80% people at risk or infected, do not become sick
Lymphatic filariasis and others	*Culex*		*quinquefasciatus*
Arboviruses	1 million people at risk in 71 countries (at least 36 million disfigured and incapacity		
Malaria	*Anopheles* sp.	*Plasmodium* sp.	600 million at risk (3–8 million deaths)
West Nile virus	*Culex pipiens* and others	*Flavivirus* sp.	Difficult to determine, most cases being asymptomatic

Eco-friendly control tools are urgently needed. In recent years, huge efforts have been made to investigate the efficacy of botanical products against mosquito vectors; many plant-borne compounds have been reported as effective against Culicidae, acting as adulticidal, larvicidal, ovicidal, oviposition deterrent, growth and/or reproduction inhibitors, and/or adult repellents.

The insect world

It is necessary to explain why most disease vectors are insects. The most important vectors are reported in Table 5.3. If microorganisms, like bacteria, are the most relevant component of biomass, insects are the predominant part of biodiversity. We are talking about more than a million described species, accounting for more than half of all known living organisms on the planet. However, the total number of extant insect species is estimated at between 6 million and 10 million. Potentially more than 90% of animal life forms on Earth are insects and these numbers increase if we consider the arthropod phylum, of which *Insecta* are the largest class. Therefore, they take myriad different forms, but to recognize an insect at a glance you must remember that they have six legs (hexapod), an external skeleton (exoskeleton), the body is divided in three parts, and the development involves a series of molts (metamorphoses). People imagine insects walking, flying, or sometimes swimming, but many insects spend at least part of their lives under water, in the larval state. Therefore, water is very important in the pathway to the adult and at larval and egg states, the insects are water-dependent and unable to move around easily. In other words, they are easy to find and vulnerable in the early stages. Insects are common in nearly all environments, although only a small number of species may be found in the oceans and in ice lands.

Table 5.3 The most important vectors in insect-borne diseases.

Anopheles sp.	• Malaria • Lymphatic filariasis
Aedes sp.	• Chikungunya • Dengue fever • Lymphatic filariasis • Rift Valley fever • Yellow fever • Zika
Culex sp.	• Japanese encephalitis

The science of studying insects is named entomology, from the Greek word ἔντομον (*éntomon*), meaning "cut into sections," an etymology present also in the Latin word *insectus*, literally meaning "cut into" and therefore "with a notched or divided body." Segmentation of the body is more or less widely present in many forms of pluricellular living organisms. Segmentation in animals typically falls into three types, characteristic of different species of arthropods, vertebrates, and annelids. Segmentation is important for allowing independence of the parts, consisting of free movements, special development of certain parts, and in some individuals the regeneration of certain parts. It is also conservation of energy by fractal repetition of the part, like in our segmented vertebral column. This is probably why the millipede, *Illacme plenipes*, presents 170 segments and 662 legs! Therefore, segmentation may result in the repetition of organs or in a body composed of self-similar units, or both. The taxonomic consequence is the classical splitting of segmented organisms into eusegmented and pseudosegmented. Eusegmented organisms are said to be comprised of "segments," as in the case of arthropods and annelids, which are said to be distinguished by their mode of formation from a posterior growth zone. When this characteristic is absent or does not affect all organ systems, there is a distinction in the generation, named "pseudosegmentation" or "metamerism." All insects have three main body regions: head, thorax, and abdomen. The head consists of the main visible parts on the head as the large compound eyes, the antenna (feelers), and the mouthparts. The thorax is the middle region of the body and bears the legs and wings, if the latter are present. The abdomen, usually deprived of outstanding features on most insects, looks like a series of similar segments. The mouth and the abdomen play key roles in insect-borne disease. In particular, the abdomen, often considered of secondary importance, is a necessary reservoir for the parasite. In insect-borne diseases, the segmentation of the vector is important for the role of each segment in the transmission and development of the disease, as well as the consequent adaptation of the parasite. Arthropods may be a good vector due to their passive role in parasitism, considering their protection by the exoskeleton and the segmentation, which creates an efficient container, but the best vectors are certainly the biting ones, because the key strategy is transmission.

Therefore, among the vectors responsible for diseases of major public health significance globally, a special place must be assigned to mosquitoes. Taxonomically, mosquitoes belong to phylum Arthropoda, class Insecta, order Diptera, family Culicidae. Culicidae, divided into three subfamilies—Anophelinae, Culicinae, and Toxorhynchitinae—accounts for 3450 recognized species of mosquitoes in 38 genera. In some cases, like malaria, the etiologic agent is localized in one genus, like malaria in *Anopheles*; in other cases, a single vector can be a vector of several diseases, like *Culex* sp. Arthropods, in addition to insects, include arachnids, myriapods, and crustaceans.

However, there is probably another similar answer regarding their prevalent role as vectors, relating to their incredible variability. Why are insects such a reservoir of biodiversity compared to any other form of organisms? Probably because they are extremely able to adapt and interact with different kinds of environments. A great deal of evidence of the special adaption capacities of insects can be seen around us. Insects are important pollinators of the most widespread and advanced plants, the Angiosperms, and contributed heavily to their success by a natural mechanism called coevolution. Angiosperms, also known as flowering plants, developed a close alliance with insects based on the characters of flowers, including coloration and flavor. In other words, the distinct and special coloration of a flower is the consequence of a relationship between that particular type of plant and that particular type of insect, a consequence of different environmental and evolutionary advancement. Modern insects appeared in the Devonian era (about 400 million years ago). In pollination, in addition to nutrition (nectars) and smell (attractant odors), colors are important as one of the distinctive signaling characters of the plant species. Flowers, as the reproductive part of the plant containing micro- and macrospores in sporangia, were already present in Gymnosperms and in the primitive Angiosperms, but not colored, and early insects (such as beetles) acting as pollinators had little vision, did not fly, and visited flowers with pale colors (white, cream, brown). The advent of Angiosperms dates to 130 million years ago, in the early Cretaceous period, and changed the scenario: flies appeared, able to fly and with modified eyes to see colored flowers, including purple and red. Later, in the Tertiary Age, the coevolution generated the sequence: wasps seeing up to yellow flowers, bees to purple, yellow, blue and even UV flowers, and finally butterflies from red to blue.

This is only one example, probably the clearest one to us, of the result of a strong selective environmental pressure and the ability of insects to respond efficiently. Insect-borne diseases are just one consequence of such efficiency. Insect vectors of diseases are numerous and varying in their characteristics as dependent on their habitats of origin. Generally, among the four stages, the first three (egg, larva, pup) need particular conditions to develop, being aquatic or needing stagnant water. Adults are small (varying in length from 5 to 13 mm), with slender and fragile bodies, a pair of narrow wings, and three pairs of long legs. They can fly for very short distances and survive for long journeys, but being light they can be transported by the wind, performing long journeys and therefore migration is not a problem. If the distance is too great, the resistant and invisible eggs can utilize any sort of transportation. Their key organ for the disease is an elongated proboscis with mouthparts adapted for piercing, being useful for feeding. This organ is also in charge of transmitting the infection.

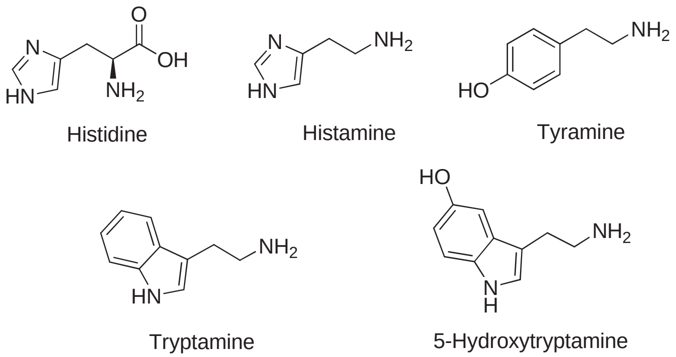
The starting point

Only a century ago, Ronald Ross discovered that malaria is caused by the bite of an infectious mosquito, evidencing that mosquito-to-human malaria transmission occurs when sporozoites from the salivary gland of the mosquito are injected into the skin during blood-feeding.

The bite of a mosquito must be considered the starting step of an insect-borne disease. It's a minor event, involving minimal parts of our body and of the vector, but it can be the start of a sequence of relevant steps causing serious damage to our health. However, we must consider what happened before the bite and that not any bite is equally impacting. Therefore, it is important to focus on the aspects (usually underestimated) that can differentiate a mosquito's bite and its consequences.

In the case of an insect-borne disease, the situation is complex and the bite is an essential but not unique part of a sequence of episodes, which are elements of cycles, and these cycles can repeat indefinitely. Before the bite, several other actions are necessary to start the infection, including a complex cycle of infection inside the vector, that precedes the further cycle in the host. The bite is the exact point of contact between the two cycles. Female mosquitoes bite humans and other animals without any intention of propagating the infection, being also victims of the disease, but in this way they obtain nutrients and energy contained in human blood. This energy is necessary to make the eggs they need to reproduce. To obtain this result, a mosquito uses the sharp tip of its straw-like mouth (proboscis or stylet) to pierce a person's skin and therefore locate the blood vessel and draw blood up through its mouth. This is the exact moment of the contact between the three actors: vector, host, and parasite. As the mosquito does this, its saliva and the prey's blood come into contact, exchanging contents. It takes some time, because of our first defensive line. The invasion carried by the bite is perceived and the body reacts, trying to stop the intruder. Thanks to an anticoagulant, the saliva stops the person's blood from clotting and, in the case of an infected mosquito, the cells of the etiologic agent of the disease (perhaps received by us) also flow into the blood, identifying the jump from a host to another. The new host, such as a human body, is usually preferred by the parasite, if not already infected. If the blood were to clot around the mosquito's mouth, it might get stuck. Mosquito bites itch and swell because of the body's histamine response. The swelling around the bite is caused by histamine, which is produced in response by the immune system. If you do not itch after being bitten, there may be something wrong with your immune system. In other words, an itch is a natural and expected reaction, showing us that our body has detected a possible harmful intruder (Lindsay et al., 2012).

We shall now focus briefly on histamine and its numerous metabolic roles, because it is not only a matter of itching. Histamine (Fig. 5.4) acts as a signaling molecule in the stomach, skin, and immune and nervous systems. Histamine-containing neurons, whose sole source is the posterior hypothalamus, innervate the whole central nervous system. They are active exclusively during walking, since first of all, this biogenic amine is one of the most important our neurotransmitters, carrying chemical messages between nerve cells. Generally, in this way signals travel from the periphery to the brain and conversely. However, histamine is also stored in the blood cells called basophils, which harbor histamine-containing special granules. Once released from its granules, histamine produces many varied effects within the body, including the dilation of blood vessels, which increases permeability and lowers blood pressure, facilitating the work of the vector. On the other side, the effect of histamine on erythrocytes is crucial for the body's health because of its role in the immune response, our main defense against the disease. Histamine is derived from the amino acid histidine by loss of the



Histidine Histamine Tyramine

Tryptamine 5-Hydroxytryptamine

Fig. 5.4 Structures of histamine and related amines acting on the central nervous system (CNS).

carboxyl group (decarboxylation). In fact, chemically, histamine is 2-(4-imidazolyl)ethylamine. The ethylamine "backbone" is a common feature of many bioamines, acting as transmitters (e.g., dopamine, norepinephrine, and serotonin). The structure is based on the general rule for neurotransmitters, like serotonin, with a flat aromatic part and a nitrogen functional group at certain distance. Despite its very simple structure, it is a bioamine that has great importance in the regulation of metabolism and appropriate response to environmental pressure. Therefore, it is distributed widely (albeit unevenly) throughout animals; it can also be found in many plants and even in bacteria. Histamine is strictly related to the nervous system, being stored within and released from neurons. Once released, histamine activates both postsynaptic and presynaptic receptors, although histaminergic nerve terminals do not exhibit a high-affinity uptake system for histamine as other neurotransmitters do, suggesting a wider functionality for this molecule.

Histamine also induces antinociceptive (pain-relieving) responses in animals after microinjection into several brain regions. In this short dissertation about the consequence of a mosquito bite, usually considered a mundane occurrence, we must consider the local reaction of bodily tissues first to injury caused by physical damage, as clearly observed in the inflammation sequence, second as agent against the infection, and third as allergic reaction. Injured tissue mast cells release histamine, causing the reaction of fluid and cells of the immune system, such as leukocytes (white blood cells) and blood plasma proteins. Together with other immune factors, they leak from the bloodstream through the vessel walls and migrate to the site of tissue injury or infection, where they begin to fight the infection and nourish and heal the injured tissues. This process is normally able to defend us, but in the case of infirmity or recurrent and massive attacks, it is not sufficient to avoid the disease. Histamine, being related to the body's immune response, can cause many symptoms of allergies, such as a runny nose or sneezing, in a person's eyes, nose, throat, lungs, skin, and/or gastrointestinal tract.

In other words, when a mosquito bite breaks the skin, a person's body recognizes the mosquito's saliva as a foreign substance. The bite causes a response of the immune system, which aims to flush out the intruder by an increase of histamine in the blood flow, meanwhile white blood cells crowd around the affected area, and this causes inflammation or swelling. There is another reason why mosquito bites itch, since histamine also sends a signal to the nerves around the bite. Nerve endings in the skin send messages to the spinal cord, telling it that histamine has been released. Histamine causes a number of immediate effects, including (if we are fortunate0 widening of the blood vessels close to the bite. Otherwise, the bite gets warm and begins to swell. Fluids escape from the single-cell layer of the capillaries, bringing with them clotting agents and white blood cells. These cells start to wrap around and digest the mosquito proteins, but there are chemicals in mosquito saliva that slow down the response of white blood cells. This effect can last up to 7 days, which is why it takes days for a mosquito bite (and the associated itch) to go away. For the same reason, sicknesses like malaria, dengue, and West Nile virus are so widespread and difficult to control. However, the chemicals in mosquito saliva only work on our immune response, and not on the process of histamine release. From the spinal cord, these signals reach the brain where they are translated into an itchy sensation.

The alerted brain send orders of reaction. Therefore, scratching is the first immediate natural reflex to an itch, and very hard to control. The itch-scratch reflex has been extensively studied, mainly in cats and dogs, and we must differentiate between an acute itch and a chronic itch. A chronic itch has very little to do with histamine, instead of the acute itch, caused by things like mosquito bites, which is regulated through histamine release. When histamines start to act, they are also pruritogens. These cause the persistent itchy sensation which typically follows a mosquito bite. The more mosquito bites you have, the more you itch. As many female mosquitoes take advantage of night hours to feed, this often leads to increased itching at night, but this depends on the species of biting mosquito. During the night when we are sleeping, the bedroom is a favorite place of female mosquitoes, not only due to our prone and unconscious state. When we are lying unprotected in our beds at night, breathing out lots of carbon dioxide and at just the right temperature, we attract many female mosquitoes, which can perform their work without interferences or hazards. Our bodies tend to have higher amounts of carbon dioxide as the night goes on, released via our lungs during respiration. Sweat and perspiration are concentrated around us and work as attractants, since CO_2 is the most efficient mosquito attractant. In this way, female mosquitoes will easily make their way to the bedroom and to us when we are resting. This also explains the sensation of being followed and persecuted by mosquitoes. More bedroom rest means more bites, and mosquito bites are favored if you are the type of person who falls into a deep sleep. If one bite releases a few granules of histamine, 10 bites will release 10 times that many. In this way, the intoxication becomes persistent and the itchy sensation will increase, although in the case of insect-borne disease, this is just a collateral effect.

A further reason favoring biting activity during the night is because in the bedroom, we have fewer distractions. The itch-scratch reflex is controlled by the brain. Mosquitoes adapt to our daily rhythms to increase their chances of getting a good meal and they can live through winter in the right environment. During the day, our brain is usually busy and distracted, but active, and it can tell us to scratch or react against the mosquito bite.

In many places in the world, mosquito-borne diseases are endemic, and at the sunset this compact army of flying vectors moves to invade and conquer any place in search of human targets. The greater the number of parasites within the salivary gland of the mosquito following blood-feeding, the more likely it is to have transmitted the disease. In other words, highly infected mosquitoes are more likely to cause infection (and to do so quicker) than lightly infected mosquitoes. This suggests that mosquito-based methods for measuring transmission in the field need to be refined as they currently only consider whether a mosquito is infected or not (and not how heavily infected the mosquito is).

Our reaction is important. The type of mosquito bite does not make a difference to the itch, although the larger the mosquito is, the more saliva is injected, and the more histamine is released. Frequent mosquito bites mean more itching. Histamine can be released over days, with multiple bites over multiple days having a cumulative effect. Whether that pesky mosquito is an *Anopheles* or a *Culex*, its bite is going to itch. An itch-free mosquito bite simply does not exist—unless, of course, we do something about it. Stopping the itch does not just mean applying a cream or taking a pill. As in all good medicine, prevention is far better than cure. A vicious circle of bites, involving histamine release, itch-scratch reflex and local inflammation, can be counteracted by use of a repellent or antihistamines, hydrocortisone creams, and ammonia solutions. However, if the mosquito was infected, the consequences are more important.

Mosquitoes have been with us for millions of years and are probably one of the most studied insects on the planet, thanks to their carrying a huge number of very serious diseases, which are still no closer to being eradicated completely—quite the opposite, again with the decisive help of humanity. Many species are learning to live indoors, adapting their behavior to our living conditions and acquiring survival strategies to overcome the chilly winter temperatures.

However, the mechanism of transmission by mosquitoes is not automatic and mosquitoes are not always efficient vectors. Novel transmission reducing drugs and vaccines are tested by experimentally infecting people using infectious mosquitoes. The importance of parasite number has widespread implications across malariology, ranging from our basic understanding of the parasite, how vaccines are evaluated, and the way in which transmission should be measured in the field. The relation between the number of parasites and infection provides direct evidence to suggest that the world's first licensed malaria vaccine may be only partially effective because it fails to provide protection against highly infected mosquitoes. It also provides direct evidence for why the only registered malaria vaccine RTS,S was only partially effective in recent clinical trials.

Leaving histamine for now, it should be noted that all this is probably just a small part of the histamine story, that connects to the arguments of this book. Histamine's influences were restricted to the simple sequence of sensorial experience-perception-acquisition-response-consciousness-memory, but it is possible that histamine and other similar bioamines are involved in other levels, including emotions.

Malaria generalities

If plague is the most impressive insect-borne disease in terms of impact and devastation, malaria was, is, and probably will be the most important tropical disease affecting mankind, and is certainly nowadays the most diffused globally. The story of malaria is interesting due to its central relevance in the progress of medicine, as well as the manifestation of resistance and the consequent need of new strategies. The word malaria is derived from the Italian *mala aria*, literally meaning bad air, as the result of the association with humid and moist areas where life is difficult and air is of bad quality. The idea was that something in the air could infect people. Nobody knew about invisible dangerous creatures. In Rome, ancient hospitals were constructed with the doors angled toward the Vatican, to allow entry of the wind transporting the Holy Spirit.

The word testifies the old origins of the disease and its ancient presence mainly in central and south Italy, but this disease, in contrast to emerging ones like Zika or Ebola, should probably be considered a case of coevolution with the genus *Homo sapiens*, still in action. In other words, species of malaria agents were probably already present in the early Tertiary period some 60 million years ago; they were derived from a common ancestor, but rapidly found ideal hosts in humans, and this coexistence is destined to continue.

The wide diffusion of this disease is based on a complicated infection and propagation strategy. Again, we must refer to the sequence parasite-vector-host. Incidence of malaria differs by area. Although 40% of the world's population can be considered at risk, most transmission of *Falciparum malaria* occurs in Sub-Saharan Africa. In this area, mainly children under the age of 5 years are most affected. The incidence of disease declines in older children because of increasing immunity. In contrast, in South-East Asia, the disease occurs more commonly in adults and the clinical features are different. However, another character of distinction of malaria, different from other vectored diseases so far mainly limited to tropical and sub-tropical regions, is the historical occurrence in temperate latitudes—probably another effect of coevolution.

Regarding epidemiology, we must consider that if there are more than 500 million clinical cases every year, only 1% of symptomatic infections may develop into severe malaria. In such cases, malaria symptoms consist of anemia, hypoglycemia, and metabolic acidosis, leading to coma or multiple organ failure, which may cause more than 1 million deaths annually.

The etiological agent

The parasite is a unicellular protozoan of some species of the genus *Plasmodium*. Protozoa are considered the precursors of animals and pertain to the Protista (or Protoctista) kingdom, which is the most complex macrotaxon, wherein a plethora of different eukaryotic organisms are located. Protista were created by a need for simplification: a eukaryote, which is not an animal, a plant or a fungus, is a Protista. In the Protista biological kingdom, there are eukaryotes very different in life cycles, trophic mode, locomotion structures, areal distribution, and organization. Among them, we can find several important parasites, affecting humans, animals, and plants. Besides malaria, a number of human parasitic diseases are caused by protozoa, such as amoebiasis, giardiasis, toxoplasmosis, cryptosporidiosis, trichomoniasis, Chagas disease, leishmaniasis, African trypanosomiasis (sleeping sickness), amoebic dysentery, acanthamoeba keratitis, and primary amoebic meningoencephalitis (naegleriasis). In all these cases, the parasite is a microbe and the mechanisms of infection and disease are quite similar, and there is no definite treatment. However, malaria wins for diffusion and importance. We must consider carefully the reason for malaria's success, which involves the congruence of several key factors. In a superorganism, the mixing of viruses, prokaryotes, and eukaryotes is now substituted by all eukaryotes, although pertaining to very different organisms: the pathogen as the protozoan *Plasmodium*, the vector as the insect *Anopheles*, and the host as humans or other animals (Table 5.4).

Table 5.4 Scientific classification of *Plasmodium falciparum*.

Dominium	Eukaryota
Regnum	Protista
Subregnum	Chromalveolata
Clade	SAR
Infrakingdom	Alveolata
Phylum	Apicomplexa
Class	Aconoidasida
Order	Haemosporida
Family	Plasmodiidae
Genus	*Plasmodium*
Species	*Plasmodium falciparum* Welch 1879
Synonyms	*Oscillaria malariae* Laveran 1881, *Plasmodium malariae* Marchiafava and Celli 1885, *Laverania malariae* Feletti and Grassi 1880

The genus *Plasmodium* includes more than 170 different species that infect mammals, reptiles, birds, and amphibians. However, only four species have long been known to cause malaria in humans: *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*, with several subspecies and varieties. *P. falciparum* is considered responsible for the majority of the morbidity and mortality attributed to malaria, especially involving the central nervous system (CNS). It is also possible that something is changing since more recently, *P. knowlesi*, which normally infects long-tailed and pig-tailed macaque monkeys, has also been implicated as a cause of human malaria in South-East Asia: Borneo, Thailand, Singapore, and parts of the Philippines.

The vector

Anopheles, like any other flying insects, are efficient vectors and by their biting the infection can be easily transferred. There are approximately 3500 species of mosquitoes grouped into 41 genera, but of the approximately 530 *Anopheles* species, only 30–40 transmit malaria in nature. The rest, even biting humans frequently, cannot sustain development of malaria parasites. They can easily be present in rural and urban habitats, needing only small quantities of water to survive and being able to reproduce in large numbers. Malaria is transmitted by different *Anopheles* species in several geographic regions, since different environments support different species. Male mosquitoes do not bite, so cannot transmit malaria or other diseases. The biting adult females are generally short-lived, with only a small proportion living long enough (more than 10 days in tropical regions) to transmit malaria. These limitations are not sufficient to restrict malaria diffusion anywhere. In fact, *Anopheles* are present worldwide except Antarctica, and we have long known that malaria is present also in temperate regions, although the vector is active only in hot seasons.

In more detail, the taxonomic studies of entomologists and geneticists have led to the knowledge of 537 species of *Anopheles* currently known and formally named (87%). Genus *Anopheles* is disproportionately divided between seven subgenera with different geographic distributions and numbers of species: *Anopheles* (cosmopolitan, 182 species), *Baimaia* (Oriental, one species), *Cellia* (Old World, 220 species), *Kerteszia* (Neotropical, 12 species), *Lophopodomyia* (Neotropical, six species), *Nyssorhynchus* (Neotropical, 39 species), and *Stethomyia* (Neotropical, five species). Four of the subgenera, *Anopheles*, *Cellia*, *Kerteszia*, and *Nyssorhynchus*, include the species that transmit human malarial parasites. In several cases of *Anopheles* vectors, complexes of sibling species were reported, like in *A. gambiae*, the most important vector of malaria in Sub-Saharan Africa, which consists of at least seven morphologically indistinguishable species. With respect to plague, malaria presents some analogies and several differences. In particular, mosquitoes guarantee a better and wider distribution, although *Anopheles* is mainly restricted to hot countries, because its species are not able to survive easily in cool weather. Owing to their impact on human health, concerning malaria and filariasis, *Anopheles* is the most studied and best-known genus of mosquitoes. As vectors, *Anopheles* mosquitoes have affected the lives of more humans than any other insect and have had an impact on human genome evolution, such as emergence of sickle cell anemia as a mode of resistance to malarial protozoa and others.

The host

In addition to mosquitoes and humans, malaria can affect several animals, such as reptiles, birds, and various mammals. Although the data must be considered with caution, considering the difficulties to obtain reliable information and the wide diffusion of the disease, it is estimated that malaria could affect 550–600 million people worldwide and cause an estimated 1–3 million deaths each year. In addition, individuals with asymptomatic malaria (carriers of *Plasmodium* species) are significantly under-identified and thus represent a large unknown transmission factor for malaria, since the majority is often undeclared or unknown. In many countries, malaria is considered endemic and in some way populations and parasites have developed some kind of mutual cohabitation.

The disease is widely distributed in subtropical and tropical developing countries, particularly in Sub-Saharan Africa, Central America and the Caribbean, South America, Central and South Asia, temperate parts of East Asia and South-East Asia, and parts of Oceania. Although the vectors are present in all these areas, the types of malaria are differentiated, since the *Plasmodium* reported above are unequally distributed. *P. falciparum* infection is predominant in Sub-Saharan Africa, South-East Asia, and some parts of the Caribbean, especially Haiti and the Dominican Republic. *P. falciparum* and *P. vivax* are concomitantly encountered in South America and the Indian subcontinent. *P. malariae* is found most commonly in Sub-Saharan Africa, but may be encountered in most endemic areas.

Malaria in detail

Every step of the mechanism of malaria transmission and disease effects has been carefully elucidated, although several aspects concerning its control are still unclear. The parasite is a protozoan, which allows a large possibility of metamorphosis, which is the main reason for the success of malaria. The capacity of adaptation of *Plasmodium* to different microhabitats is the key. The term "metamorphosis," meaning transformation, modification, and change of form, may have different interpretations in biology, depending on the discipline. In botany, the term is used in the case of alteration or degeneration of an organ or a part of a plant in which tissues are changed, like a leaf changed into a thorn, causing a definitive change. Thus, the spinification of leaves in succulent plants, like Cactaceae and Euphorbiaceae, is a metamorphosis of an organ into something adapted to arid and hot conditions. In zoology, the term is strictly utilized for the process of transformation from an immature form to an adult one in two or more distinct stages, like in an insect or amphibian. The change can be radical in these organisms, whereas in humans we have mutation but not mutants. However, in the human fetus there are a series of transformations and in our genome there are evident traces of the sequences of other organisms.

During the occurrence of malaria, *Plasmodium* undergoes a programmed series of metamorphoses, selected to obtain the best adaptation to host cell environments. We must remember that each organism can be identified by and in its DNA. Our life starts with parental DNA and ends with our own

DNA if we have progeny. Life starts from another life, through nucleic acids transmission. Evolution is concentrated on adaptation to the environment and homeostasis of this unique molecule. DNA is an extraordinary macromolecule, being the most complex that we know. However, it has a handicap: this molecule is delicate and needs protection, consisting in an involucre, more or less complex, like a cell or a body, in order to work and survive. However, in the viruses only a protein involucre is present. Among the consequences, the metamorphosis of the vector and the parasite means that, despite the radical changes of form, the individual is the same, being identified in the genome. Emerging from temporary packaging, the DNA can revive, maintaining its identity. The second advantage consists of a smart alternation between different modes of reproduction, including a turnover between asexual and sexual ones. Furthermore, *Plasmodium* is able to remain dormant inside the host, waiting for an opportune moment to diffuse and maintaining a situation of misleading equilibrium of the disease. In comparison with the catastrophic explosions of plague, the situation in malaria can be maintained at a minimal level for long periods, allowing the possibility of endemic disease and recrudescence. Finally, the symptoms and health effects of various malaria diseases are the results of previous differences, and include also important targets, like the nervous system.

The main consideration is that the parasite is able to conform not only to the different hosts, but also to the hosts' different microhabitats. *Plasmodium* jumps from mosquitoes to mammals and vice versa, and adapts its morphology in harmony. The reason for these changes is the need to ensure and optimize alimentation for the Protista, as any other living organism. In particular, the female mosquito is looking for energy, and the most profitable and tempting sources of energy are our red blood cells and its precious content, hemoglobin. Generation of a plethora of new lives urgently requires great quantities of energy. Our blood is a valuable reservoir of energy; the female vector feeds on our blood to aliment its eggs and the microorganism feeds on blood cells to fuel its frantic meiosis.

The whole process of the infection and reproduction is quite complex, comprehending two cycles, one in the vector and another in the host. Let us now concentrate on the human cycle, and therefore on malarial disease. When the female *Anopheles* mosquito bites someone, infection is transmitted from the saliva of the vector to the patient for its blood, wherein *Plasmodium* cells, as sporozoites, follow the bloodstream. Only a few sporozoites are injected directly into the blood vessels via the mosquito bite. Therefore, the parasite first needs to reproduce. At that time, *Plasmodium* is a sporozoite, the best form for rapid and successful reproduction, but it is also protected by a solid cell wall during the dangerous journey from one organism to another. The sporozoites efficiently leave the skin, localize in the liver, and invade hepatocytes. Within 8 h, sporozoites are carried rapidly to the liver where they multiply asexually and in approximately 7–10 days they are able to transform, becoming liver schizonts.

The third phase of the *Plasmodium* path involves the blood after the liver step. When they mature, these schizonts release thousands of merozoites into the bloodstream, where they cause the infected hepatocytes to burst. Inside the sporozoite cell, the single nucleus generates many other similar nuclei. The cell then explodes, liberating its content when nucleu have become cells. One single *Plasmodium* sporozoite in one liver cell multiplies into tens of thousands of exoerythrocytic merozoites, each of which is able to invade a red blood cell, initiating the stage of the infection that causes the fever of malarial disease. However, if the conditions are not appropriate, alternatively dormant hypnozoites are produced by *P. vivax* and *P. ovale*. In this way, the whole potentiality of the parasite can be totally maintained and preserved, ready to act in better times.

The merozoites are the best form to benefit from the treasured energy contained in the erythrocytes. Merozoite's invasion of red blood cells involves multiple interactions and events to satisfy the conditions of the new microhabitat. Merozoites first recognize their target by contact, then reorient and attach to surface receptors on the erythrocytes, and finally penetrate and infect individual erythrocytes (Fig. 5.5). A substantial processing of merozoite surface proteins occurs before, during, and after invasion. Merozoites possess a particular sensitive fibrillar coat of surface proteins. This coat appears remarkably during the first phase of the erythrocytes invasion and allows the merozoite to recognize the target, relaying the more active merozoite (Fig. 5.6).

The blood stage of *P. falciparum* merits a little focus in consideration of the further arguments. Inside the red cell, the bacterium utilizes the host's hemoglobin as a food source, to obtain the amino acids derived from proteolytic digestion for the parasite's biosynthetic requirements. Hemoglobin degradation involves several proteases. Once the denatured globin is produced, a cysteine protease, falcipain, degrades this denatured globin, which by the action of plasmepsins is further degraded into small peptides by other proteases until the final production of amino acids. During this process, large amounts of free nontoxic heme are released from hemoglobin as a by-product. The released heme is autoxidized into hematin, hemin, or aquaferriprotoporphyrin IX, consisting in a ferric form (Fe(III)) that is a

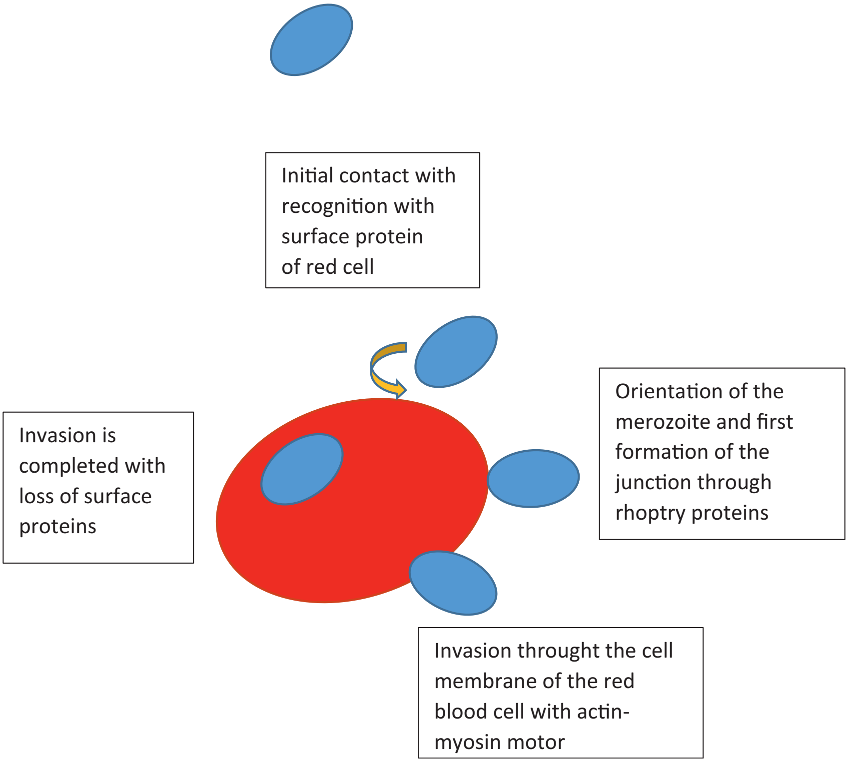


Fig. 5.5 The process of infection of the blood cell by merozoites.

highly toxic product for the parasite, inhibiting vacuolar proteases and damaging its membranes. In practice, there is a danger of autointoxication by the parasite during its alimentation and therefore a detoxification of heme is necessary for the survival and growth of malaria parasite. Enzymes convert heme into hemozoin, popularly known as malaria pigment, and consisting of a dimer of heme units linked through an iron-carboxylate bond. In the dimer, a bond is formed by the linking of central ferric iron of one heme unit with the propionate side chain of another heme. This pigment is inert in the parasite and released into the host blood supply after infected erythrocytes burst open at the end of the parasite's life cycle. Many researches have evidenced the capacity of quinoline antimalarial drugs to inhibit formation of hemozoin and therefore limit parasite growth and reproduction.

The cell surface receptors necessary for attachment are specific for the *Plasmodium* species. For example, *P. vivax* attaches to the red blood cell via a receptor related to the Duffy blood-group antigen. Thus, individuals

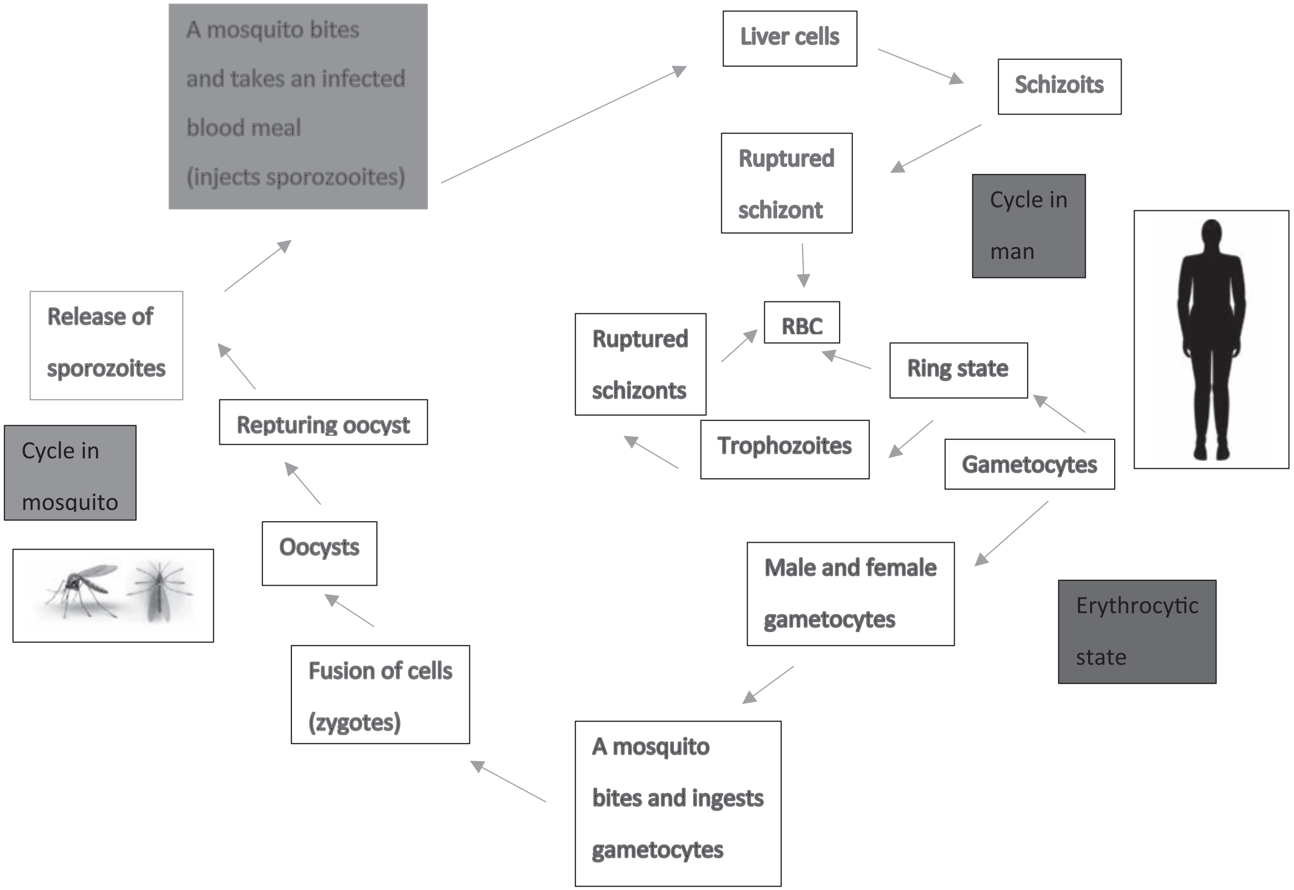


Fig. 5.6 The malaria cycles.

from West Africa, who generally have Duffy-negative blood, are resistant to invasion and infection by *P. vivax*. Even now, *P. vivax* infection remains uncommon among indigenous West Africans.

Once inside, merozoites reside in a vacuole of the host red blood cell, where they transform in the trophozoites. The trophozoites are able to feed efficiently on hemoglobin. Inside the vacuole, another transformation: the early trophozoite manifests as the classic ring form, then the trophozoite gets fat, and grows to occupy most of the erythrocyte's lumen. After about 24–36 h, it enters a second stage of asexual division, schizogony, to form an erythrocytic schizont, each containing 12–24 merozoites per infected erythrocyte. In comparison with the sporozoite, the merozoite is able to prepare the conditions for the passage to sexual reproduction. This reproduction is necessary to maintain the parasite for longer periods and to improve its genome.

The time interval between mosquito bite and entry of merozoites into the bloodstream is about 10–14 (range: 7–28) days. This is a latent period, known as the prepatent period. Continued asexual replication in the bloodstream through repeated cycles of maturation and rupture of red cells with release of merozoites eventually results in symptomatic infection. During this process, a fraction of the merozoites undergo sexual differentiation and develop into sexual forms called gametocytes, which produce no symptoms themselves, but may circulate for a prolonged period of time.

There are different types of malaria with different symptom of the disease. *P. vivax* and *P. ovale* generally have a predilection for young erythrocytes, while *P. malariae* infects old cells. For this reason, these three species seldom manifest a parasitemia greater than 2%. In contrast, *P. falciparum* infects erythrocytes of all ages and can therefore manifest with parasitemia levels often above 5%. Relapse, recurrence, and recrudescence are three important aspects of malaria, which are well known by the patients in case of untreated malaria. For malaria caused by the two relapsing malaria—*P. vivax* and *P. ovale*—treatment with a blood schizonticide invariably results in an immediate response. In relapse, a resumption of replication of previously dormant hypnozoites in the liver starts the cycle again, with development into pre-erythrocytic schizonts that produce merozoites, and these reinvade the bloodstream. A new inoculation of sporozoites from a mosquito bite may generate a recurrence. Following a single exposure/mosquito bite, the malaria-naïve patient may perish during the initial disease or develop both a humoral and cellular immune response, which after recurrent infections could lead to a phenomenon known as premunition. A recurrence is called a recrudescence if it is caused by the persistence of blood forms in small numbers between attacks. Recrudescence may occur over a period of many years, causing a replication of the disease's symptoms.

In 1970, the WHO officially declared Italy free of malaria. However, from 1985 hundreds of new cases were registered yearly, increased to 1000 by the year 2000, with seven deaths. These cases concerned import diseases with Italian citizens travelling for tourism or migrating, but also diseases contracted in Italy.

As we have seen, the situation differs between developed and developing countries, but there is a common feature: both situations are in evolution.

The state of the art of malaria

Malaria is the most important parasitosis and the second most infective disease in the world for morbidity and mortality, after tuberculosis. It is considered endemic for 40% of the population in subtropical and tropical areas, at altitudes below 1800 m. Officially, every year 10,000–30,000 European and American travelers, using air or sea transportation, are affected by malaria. However, these data must be considered underestimated since most of them solve the disease with autodiagnosis, assuming antimalarial drugs.

Malaria is popularly known for the typical cyclic intermittent fevers, corresponding to cycles of reproduction of the parasite. The infection by *P. falciparum* is known as malignant tertiana or tropical disease, whereas that from *P. vivax* and *P. ovale* is the benignant tertiana, and finally that from *P. malariae* is the quartana intermittent fever malaria. In malaria, the terms "tertiana" and "quartana" refer to the intermittent occurring of the symptoms, like the fever. However, these terms are misleading, considering that only a minimal number of cases present intermittent fever. Every 48 h in tertiana (meaning every 3 days) or every 72 h in quartana (meaning the 4th day), respectively. The fever is caused by the presence of various generations of the parasite in the host blood. However, the initial symptoms of malaria are similar to those of common flu, such as cephalic pain, shivers, alternant feeling of cold and hot, and general illness and discomfort. As already considered, a *P. falciparum* infection may cause kidney distress, lung edema, endocranic hypertension, until coma and final *exitus*, consisting in the death of the patient. Death is a consequence of damage by the package of the parasited cells inside vital organs, in particular in the case of the very dangerous cerebral malaria (Chen et al., 2000).

Cerebral malaria is the most severe neurological manifestation of cases of malaria infection by *Plasmodium falciparum*. The clinical syndrome of cerebral malaria is characterized by coma and asexual forms of the parasite on peripheral blood smears. The incidence is considered to be 1120/100,000 each year in endemic areas of Africa, with 575,000 children in danger of developing cerebral malaria annually and peak incidence in the pre-school period. Therefore, African children under the age of 5 years are most affected and the incidence of this disease declines in older children with increasing immunity. The coma develops suddenly, often with seizure onset, following 1–3 days of fever. A few children develop coma following progressive weakness and prostration and other important symptoms. In adults, cerebral malaria is part of a multiorgan disease. Patients develop fever, headache, body aches, and progressively delirium and coma, but compared to African children, papilledema seizures and retinal changes are less common and time to reach coma is slower. Mortality is high, but surviving patients can also present pathogenesis with relevant brain injury and long-term neuro-cognitive impairments. The clinical symptoms appear rapidly after the infection and are attributed to parasitized red blood cells sequestered in cerebral microcirculation.

However, recent reports suggest that the incidence of severe malaria is on the decline, as in general for malaria. Recent data about current incidence of malaria can be obtained from the WHO World Malaria report (WHO, 2019). Considering the geographic distribution and the reported severe cases, it was suggested that in many areas where malaria is endemic, as a consequence of repeated infection, people are able to develop a high level of antibodies, generating a form of resistance to the infection. Another interpretation of the phenomenon considers an evolution of the parasite toward a less virulent form, allowing an increased resistance to survive together with the host. This is an important aspect considering the future development of the disease.

As a matter of fact, malaria is widely spread, but neither its distribution nor its consequences are homogenous. Central Africa is most affected by malaria, so let us consider Nigeria. Regarding malaria in this country, Dr. Elvis Eze, from Nigeria but currently working in Bart's Hospital in London, witnessed first-hand the malaria presence in Nigeria, observing that the disease killed more than 100,000 people in 2016 alone. He stated that "In Nigeria you never escape malaria." However, Nigeria is arguably one of the leading countries in Africa and it is considered an emerging country. In fact, despite the challenges caused by the insect-borne diseases and a never-ending bloody war between the Muslim North and the Catholic South, and the expectation medium age of 18 years, in the last 15 years the population rose to 30 million, and in the next few years Nigeria is predicted to become one of the most populated countries in the word. On the light of the occidental parameters, this can be considered a paradox, in consideration also of the bad life conditions of most population in Nigeria and the incidence of diseases. On the contrary, in developed and advanced countries, where wellness is ensured, the population is decreasing everywhere due to the low birth rate. Looking at these data, malaria is not the most important problem for Nigerian people and similar considerations can be used for neighboring countries. In any case, malaria cannot be considered the sole reason for the current migration from Central Africa to Europe.

Signs from the past

Molecular biology gives us precious information, looking for ancient traces in fossils of our parasitized forefathers—in this case, through analysis of genomes of the protozoaires of the Laverania family, whose members could be considered the ancestors of *Plasmodium falciparum*, or in any case closely related to *Plasmodium* (Otto et al., 2014). Dr. Thomas Otto reports:

"Using the parasites' genomic data, their family tree was constructed and identified major genetic events that led to their emergence (Otto et al., 2018). The movement of a single cluster of genes was an early crucial event that enabled the malaria parasites to infect the red blood cells of a new host species. After reconfiguring and fine-tuning the repertoires of genes that interact with the host and the vector, the parasites were able to establish long-term transmissible infections in humans."

The first evidence of malaria parasites was found in mosquitoes preserved in amber and dated to the Palaeogene period, meaning they are approximately 30 million years old (Poinar, 2005). This founding does not mean the presence of malaria as we know, since human malaria, which likely originated in Africa, coevolved for a long time passing through a series of its hosts, including nonhuman primates. In fact, protozoa, responsible for malaria, are diversified into primate, rodent, bird, and reptile host lineages.

Probably, everything started 50,000 years ago, although the first real evidence of the presence of malaria parasites dated 4000–6000 years ago, and this is probably in connection with the first great human concentrations. The research was able to design the genealogical tree of the Laverania until the appearance of the cluster of genes responsible for the transmission of malaria to humans. *Plasmodium falciparum* has a common ancestor with the gorilla parasite *Plasmodium praefalciparum*; however, the latter is not capable of establishing repeated infection and transmission in humans. Otto's team was able to generate multiple genomes from all known Laverania species. The complete genome sequence of the closest ancestor of *P. falciparum* enabled researchers to estimate the timing of the beginning of speciation at 40,000–60,000 years ago.

According to the paper published in *Nature Microbiology* by the researchers of the eminent Wellcome Sanger Institute in Britain, *P. falciparum* was the only parasite of the Laverania family able to adapt its transfer successfully from gorillas to humans as reported by Dr. Matt Berriman (Otto et al., 2018): "We sequenced the genomes of all known species in a family of malaria parasites that gave rise to the deadliest form of human malaria. We estimated when *Plasmodium falciparum* and its relatives diverged and found evidence that the recent expansion of modern humans created the home in which the parasites irreversibly evolved into a human-specific form." This research is important and the obtained indications have current relevance. Many infective dangerous diseases can remain sequestrated into a preferred host for a long time, but environmental forces can generate some genetic changes and induce the jump that infects mankind.

Today I have two maps on my desk. One contains the diffusion of *Homo sapiens* from a point in Central Africa to everywhere. Geneticists were able to sign pathways and dates, evidencing the progressive spread of our species to any possible part of the planet. Our ancestors were brave explorers and many lands, such as America or Oceania, were discovered many times before the dates officially reported in history. These explorers could trust only a few natural instruments and their insatiable thirst for knowledge. The second map is the phylogenetic tree of our species. This tree presents many branches. At the end of each branch there is an ancestor more or less similar to us, but only one contains a surviving species, since all the others were extinguished, including, very recently, Neanderthal man. The unique remaining species is *Homo sapiens*. Perhaps there is a connection between the two maps. The disappearance of the other human forms and the persistence of only one is a peculiarity. It is very unusual in comparison with the genealogy of other species, otherwise Darwin never could have speculated on the origin of the species, working on the minor differences between birds of nearby islands in Galapagos archipelago. What caused their extermination? And more important, *who* was responsible for their disappearance? The reply is: we are the ones responsible. Our ancestors lived in different regions, diversified by habitats and separated by geographic barriers. In principle, all the conditions were present for them to continue their evolution pathway. The explanation is therefore probably in the other map. *Homo sapiens* was an insatiable explorer, never satisfied with his current conditions. An eternal migrant, once arrived in a place, he first used to kill off the autochthonous people living there, save part of the women, conquer the territory, and utilize its sources. Later, as soon as the situation was stabilized, with villages and organization, a new vanguard started to explore and conquer new places. The species *Homo sapiens* was obsessed by an unsatisfied desire for new experiences and knowledge, and a continuous need to travel. As Ulysses in Dante's *Inferno* states: "Ye were not form'd to live the life of brutes, but virtue to pursue and knowledge high." Nowadays, practically every part of the geography of our planet has been visited and reported on, and we are striving to go to Mars, even if we expect to find there only dust and stones. And we have yet more worlds to visit and explore. Many secrets are waiting, from the subatomic world to the magnitude of the universe. Many experiences are still available to extinguish and satisfy our thirst for new discoveries. Scientists are pushed by such a desire, and some, probably the most imaginative ones, like to explore through time, looking for traces of past events, to explain the present and predict the future. In genomic studies, the traces of genome changes are followed and connected with the spread of the disease (Sallares et al., 2004) and the migratory pathways (Rolshausen, 2019; Teri et al., 2009; Birney et al., 2007; Jackson, 2015).

Again by analysis of DNA bodies in graves, we know that malaria was present in Italy at least 2000 years ago. DNA is a delicate molecule, being made by the junction of monomers, in this case consisting of the nucleotides. Furthermore, the conformational configurational structure can be degenerated by several agents. Even the nucleotides are made up of three different components. In any junction, hydrolysis, the most common reaction in metabolism, can affect the stability of a nucleic acid. Therefore, it is very difficult for DNA to remain unchanged during centuries, unless it can benefit from a special protection.

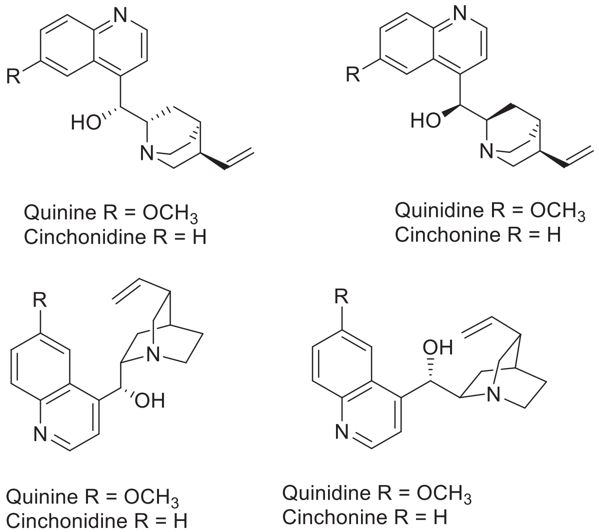
In the pulp inside human teeth, genomic traces of *Plasmodium falciparum* were determined, confirming many reports that malaria was endemic in Imperial Rome. The reports of that time state that several districts of the town were considered highly dangerous because of the disease, facilitated by the presence near the town of a wide swampland, the Agro Pontino, an ideal reservoir for mosquitoes. The situation continued without changes until 1623. In that year, there was a conclave in Rome to elect the new pope.

A conclave in Rome was considered by the cardinals and their entourages as a calamity, since in such occasion many of them were likely to be victims of the malaria. However, at the conclave the Spanish cardinal Juan De Lugo distributed Cinchona bark, saving the lives of the cardinals. Afterwards, the bark was utilized in the Arciospedale di Santo Spirito in Rome with great success. The bark of Cinchona trees was an old and popular remedy in Peru, where the trees are common in the mountains of Andes (Baliraine et al., 2011). The name *Cinchona* for the genus was created by Linneus in 1742 in honor of Countess Cinchòn and her story linked to malaria. The story goes that when she was in Lima in the 12th century after her husband, the viceroy, had been nominated by the King of Spain to rule in those distant conquered territories, the countess fell ill with a terrible fever. No remedy could be found, until an old Peruvian servant used the bark of a local tree to save the countess. Therefore, the remedy became known as "countess's bark." In fact, the *cascarilleros*, populations of some tribes of the mountains of Andesm used to collected the bark, which was well-known in local popular medicine for its antipyretic properties. The *cascarilleros* (*cascara* means bark in Spanish) used to keep secret the place and type of plant used, so that the bark was for a long time veiled in mystery. According to some reports, the origins of the bark was kept secret by the *cascarilleros* until one of them was corrupted by Dutch merchants. The traitor paid with his life, but the seeds crossed the world, reaching Indonesia, where hybrid Cinchona trees, carefully obtained by agronomists, produced more active barks.

The story of Countess Cinchòn is a beautiful episode, a fascinating example of ethnopharmacology adequate for the birth of a potent and unique remedy, as described to Linneus by Le Condamine, but the problem is that it is not true. The viceroy had two wives; the first never left Europe and the second died in Panama during a journey to South America. However, another name for the bark, "Jesuit's bark," is more credible, since this religious order was fundamental to the diffusion of the remedy, as it was for many other vital herbal drugs. The Jesuits noticed that the Indians in Peru used to chew the bark to prevent shivering with cold and to reduce labor pains in women (a utilization recalling that of coca leaves). They also considered the bark useful to cure shivering caused by malaria and brought the bark back to Europe, where at that time the disease was a significant problem, with a large part of its population suffering from malaria. From that time, correct dosing of the bark or extracts of the bark was evidenced to cure several cases of malaria. It is noteworthy that the first effective drug against malaria came from South America, a continent in which malaria had not been a relevant problem before the arrival of the Europeans, and from trees growing in high mountains where both the parasite and vector are absent. This means that the resources of plants cannot be rationalized by our rational approaches, but the traditional use represents an important starting point for scientific evaluation of natural drugs.

In 1643, Cinchona bark was first officially mentioned in medicine as being used for the treatment of fever in Europe, and in 1677 it was included in the London *Pharmacopeia* as "Cortex Peruanus." In the 19th century, the bark of Cinchona trees was also known in all Europe as a raw material to obtain a powerful elixir, and considered as a panacea against fever and other diseases, including the famous mysterious vine used by the English James Talbor (or Tabor or Talbot in France). Talbor was a questionable personage who acquired great fame for his miraculous cure of malaria using a mysterious elixir. He became very famous when he was asked to intervene at the bedside of important personalities such as Charles II of England, the Dauphin of France, and the Queen of Spain. It is believed that Talbor's elixir contained also opium, as in another pharmaceutical panacea, laudanum, but the main ingredient, as revealed after Talbor's death, was Cinchona bark. Nowadays the elixir of quinine is also produced and considered a potent tonic. Cinchona bark extracts are used as an ingredient in many aperitifs, digestifs, and soft drinks, daily consumed everywhere. Quinine is a flavor component of tonic water, bitter lemon, vermouth, and cocktails. The USA Food and Drug Administration (FDA) limits quinine in tonic water to 83 ppm, due to its side effects. Quinine is used instead of strychnine as a standard substance for a bitter taste (threshold of sulfate salt: 0.000008) in gustatory physiology.

About 35 quinine type alkaloids have been reported from *Cinchona* species. The antimalarial activity of Cinchona bark must be assigned to four complex quinoline alkaloids, all used as a mixture together. The four alkaloids, i.e., quinine, cinchonine, cinchonidine, and quinidine, are present in different yields in various species and varieties (Fig. 5.7). The most utilized species are *C. calissaya* (known as yellow quine), *C. succirubra* (red quine), *C. ledgeriana* (gray quine), and their hybrids, also differing in alkaloid contents. The structures of these alkaloids are based on a quinoline moiety attached through a secondary alcohol linkage to a quinuclidine ring having a vinyl group. The first part is bicyclic and aromatic, whereas the second is an unusual heterocyclic system, in some way recalling the tropane unit of some Solanaceae species. The stereogenic center at C9, consisting of a secondary alcoholic group, affords two pairs of epimers: quinine and cinchonidine



Quinine R = OCH_3
Cinchonidine R = H

Quinidine R = OCH_3
Cinchonine R = H

Quinine R = OCH_3
Cinchonidine R = H

Quinidine R = OCH_3
Cinchonine R = H

Fig. 5.7 The structures of Cinchona bark's main alkaloids. In the figure, the same structures of natural alkaloids are presented in two molecular writing forms. In such way, some 3D particulars can be evidenced.

versus quinidine and cinchonine. Therefore, quinine and cinchonidine are (−)-isomers, and quinidine and cinchonine are (+)-isomers. However, quinine and quinidine each contain a methoxy group, which is absent in cinchonidine and cinchonine.

Quinine has been the leading compound for the development of a large number of antimalarial compounds, where the functional groups, like vinyl and hydroxyl, have been used to modify the structure or add other units. Quinine is less potent as an antimalarial but less toxic than quinidine. It has schizontocidal and gametocidal activities against *P. vivax* and *P. malariae*, but not for *P. falciparum*. Therefore, quinine has been used as a suppressive and therapeutic agent, but not as a prophylactic agent. In any case, widespread use of quinine is limited due to toxicity and adverse effects. The continuous use of quinine causes side effects, which can be severe in some cases and include cardiotoxicity, visual disturbance/blindness, deafness, convulsions, and hypoglycemia. The side effect consequent of an overdose of quinine is named cinchonism, and this can be expressed through various symptoms including rashes, headache, confusion, and vomiting.

The most widely accepted hypothesis of quinine's action is based on the well-studied and closely related chloroquine. This model involves the inhibition of enzyme heme polymerase avoiding hemezoin biocrystallization.

During the feeding of the *Plasmodium* inside red blood cells, free cytotoxic hemes are produced and they accumulate in the parasites. Free hemes are toxic to the parasites, causing their deaths. However, using heme polymerization, *Plasmodium* is able to convert the toxicity to a form of protection by the heme detoxification pathway, which facilitates the aggregation of cytotoxic heme. Chloroquine's mechanism of action, also hypothesized for quinine, against *Plasmodium falciparum* is theorized by interfering with the parasite's ability to dissolve and metabolize hemoglobin. When the parasite enters red blood cells and starts to feed hemoglobin, the heme (the porphirinic portion) is realized.

Heme is toxic to *Plasmodium*. Therefore, more feeding by the parasite means also more production of toxins, but the *Plasmodium* is able to solve the problem by the polymerization of the hemes, obtaining a detoxification. As with other quinoline antimalarial drugs, quinine's mechanism of action has not been fully resolved. Therefore, the therapeutic effect of chloroquine is based on the fact that when it reaches the food vacuole of the parasite, it binds hemin (a toxic compound) and inhibits conversion of this compound to hemozoin (a nontoxic compound). Accumulation of hemin inside the food vacuole of the parasite results in the parasite's cell death.

In another interpretation, quinine may target malaria's purine nucleoside phosphorylase enzyme, subtracting energy from this key molecule. Thus, we are reporting two hypotheses: the one purely molecular based on the detoxification process and the other one focused on energy. In any case, the enzyme's key metabolic role must be considered. In consideration of the structural differences between quinine alkaloids and diterpene artemisinins, it has been necessary to explore other possible mechanisms of action.

For centuries, the complex of Cinchona alkaloids was the only available remedy to treat malaria and relieve its symptoms, in particular as an antipyretic. In Italy, before the Second World War, quinine was considered strategic for the health of the population and was therefore nationalized along with tobacco and salt. It was easy to find and buy quinine, since quinine tablets were sold by tobacconists or directly distributed by the state. In the same years, Agro Pontino was reclaimed by the Mussolini government, finally releasing Rome from malaria after thousands of years. Now it is a fertile land where valuable varieties of artichokes and tomatoes are produced.

Malaria and quinine were linked for centuries, and Cinchona trees were cultivated in many tropical sites to satisfy the enormous demand for quinine. Despite several reported syntheses, Cinchona trees remain the only economically practical source of quinine. Most natural products cannot be reproduced by chemical synthesis, due to the number of chiral centers usually present. A popular solution is hemisynthesis, consisting of the transformation of precursors present in the plant by a few steps. As a matter of fact, although many organic syntheses of quinine are reported and available, even if the ratio of any passage is high, more than five passages means a unsuitable cost of production. Another approach is when the structure of a natural product is used as a template for compounds with similar or increased activity.

However, on the basis of their quinoline structures, useful medical drugs were synthetized including chloroquine, which ensured adequate treatment for a long time. Until the early 1900s, quinine was used exclusively against malaria, until chloroquine and mefloquine were synthesized and introduced on a large scale. Chloroquine was the drug of choice because of its low cost and absence of quinine's side effects. In consideration of the subjects which should be treated by the drug, the cost of the remedy is a crucial factor. According to a 2002 study, prices for tablet treatments can be compared as follows: $0.09 chloroquine, $2.73 quinine, $5.04 mefloquine, and $5.34 artemisinin. The success of the chloroquine pill is second only to ASA (aspirin). Despite several collateral effects, chloroquine has been widely used by people living in or visiting places where malaria was endemic, confident in its power to prevent or limit malaria. The concomitant action of DDT on the vector and chloroquine on *Plasmodium* dealt an almost mortal blow to malaria—until the counteractions of *Plasmodium* and the mosquito.

The mechanism of action of antimalarial drugs is still largely unclear, since most of them were identified on the basis of their potent antimalarial properties in phenotypic screenings, without a rational design to interfere with a specific molecular target, but rather on the basis of efficacy. During the Second World War, the best cultivations of Cinchona trees were in Indonesia, thanks to the excellent work on hybridization carried out by the Dutch growers between *C. calyssaya* (yellow quine), and *C. succirubra* (red quine). During the Second World War, the United States was fighting in the Orient against Japan. The shortage of the Cinchona bark, due to the difficulties of obtaining the drug from the production places, became a serious problem, to treat the American soldiers, which came from the other part of the ocean and totally accustomed to other situations and life conditions. Under wartime pressure, research into synthetic production was undertaken. In 1944, the chemical synthesis of quinine was accomplished by the American chemists R.B. Woodward and W.E. Doering, two legendary pioneers in the story of organic chemistry. The synthetic approach to antimalarial drugs considered the need of the quinoline core along with amine groups, just like quinine. Quinine was used as a scaffold for production of synthetic antimalarial drugs, such as mefloquine, amodiaquine, and mepacrine. However, natural products are too complex and none of the syntheses can compete in economic terms with isolation of the alkaloids from natural sources. The synthesis must be stereo-controlled, in consideration of all four stereogenic centers of quinine, and needs an asymmetric catalytic method, as well as the difficult separation of the mixture of epimers derived by the synthesis. In any case, the best afforded solution was a production of synthetic quinine on 16 chemical steps.

Under the pressure of the war and the military command, English, American, and Australian researchers failed to follow scientific methods, hoping for a stroke of luck instead. They worked on the quinine structure, making a large number of derivatives and chemical similarities. They tested 16,000 compounds before finding a useful trace for a safe and efficient antimalarial drug. They knew that in 1856 a young chemist called William Henry Perkin, when attempting to synthesize quinine, had obtained mauveine, which was the world's first synthetic dye. The synthesis was accidental; Perkin was on his Easter holidays and performing some experiments in the laboratory in his apartment in East London. It was an example of pure serendipity, surely not the only one in science. Becoming interested in dye chemistry, Perkin obtained an important result with the synthesis of methylene blue, derived from mauveine (Fig. 5.8). Methylene blue was not efficient as an antimalarial, but it was useful to visualize the parasite. Nowadays we still use this reactive to evidence cells in students' exercitations and in particular to visualize microbes with a microscope in microbiology and plant cells in botany.

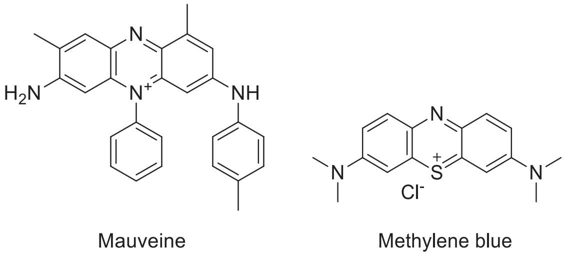


Fig. 5.8 Structure of mauveine and methylene blue.

The next story is important, wherein we can try to follow the fundamentals of modern medicine, the same ones that are still the basis of the current medical approach to treat insect-borne diseases such as malaria. Following the development of the theory of the "magic bullet," it is necessary to understand the importance and the limits of the "classic" medical approach in the fight against insect-borne diseases, as well as the central role of the immune system.

Among problems that medicine had to solve in the development of efficient drugs against insect-born diseases, the visualization and identification of parasites were decisive steps. Here we find another connection between malaria and blue methylene, and to explore it we must move to Germany between the end of the 19th century and the beginning of the 20th. It is a period of great importance for modern medicine, with the birth of chemotherapy, also known as the theory of the magic bullet. This term is a metaphor for a single chemically and physically definite molecule able to influence our physiology in order to restore homeostasis—in other words, good health—and seek out specific disease-causing agents. Direct consequences of this approach are modern-day pharmaceutical research and the relevance of the body's immune response. A vital protagonist in this radical change, and a pioneer of the main basis of our medical therapy, was the German biochemist Paul Ehrlich (1854–1915), who received the Nobel Prize in Physiology or Medicine in 1908. Ehrlich shared the Nobel Prize with Élie Metchnikoff for their separate researches on the body's immune response (Winau et al., 2004; Gensini et al., 2007). On that occasion, Ehrlich explained his chemical theory about the formation of antibodies, which are our best way to fight the toxins released by bacteria, and therefore the most effective way to obtain a medicine against vector-borne disease etiological agents by serum antitoxins and chemotherapy. Ehrlich was initially interested in a method to fight malaria by killing the evasive microscopic organism responsible of the disease. However, at that time bacteria were still practically invisible and mysterious, and it is much easier to fight an enemy if you can see it. To understand how Ehrlich reached the result of evidencing the parasites, we must consider his scientific formation. An example of a pathway full of serendipity, but also rich of geniality and capacity of treasuring any experience.

Ehrlich was born near Breslau, on the continually disputed border, at that time in Germany and now known as Wrocław, Poland. After becoming a medical doctor at the local university, and experiences in Strasbourg, Freiburg im Breisgau, and Leipzig, in Breslau he worked at the laboratory of his cousin, a pathologist called Carl Weigert, where he was fascinated by the pioneering use of aniline dyes as biological stains. Later, via his investigations at the Charité Hospital in Berlin he was able to demonstrate that selected dyes react specifically with various components of blood cells and the cells of other tissues. These studies were useful to utilize dyes for therapeutic properties to determine whether they could kill off disease-causing microbes. Among the utilized dyes were blue methylene and pyrrole blue, obtained by the condensation of pyrrole with the tetramethyl-diammidobenzophenol. First, he obtained promising results using methylene blue to kill the malaria parasite. Two other pioneers already have opened the pathway for immunology and parasitology: Louis Pasteur, with the development of the first vaccines, and Robert Koch, with his works on tuberculosis and his subsequent cure with tuberculin therapy. As consequence of the first obtained results, Ehrlich was invited to work at Koch's Institute for Infectious Diseases in Berlin. However, it is necessary to consider that Pasteur's vaccine and Koch's tuberculin were obtained from weakened bacteria, and this approach, albeit the expectative at that time were enormous, is not universally useful for microorganisms, as indicated by the difficulties to develop a vaccine against malaria. Ehrlich's strategy was based on new serum therapies, or cell-free blood liquid, extracted from the blood of naturally or artificially immunized animals to induce immunity in mankind, developing the of "antitoxins" to explain the immunizing properties of sera.

During his research, Ehrlich was in search of a chemical explanation for the immune response. In his theory, living cells and dye molecules are able to interact because both have side chains, consisting of a shorter chain or group of atoms attached to a principal chain in a molecule. In the case of dyes, the side chains are related to their coloring properties and can link with particular toxins. A cell under attack by foreign bodies produces more side chains, in the tentative of locking in the foreign bodies before they are in condition to damage the host cell. Later, the "extra" side chains break off and become the defense antibodies of the immune system, circulating throughout the body in search of toxins. It was these antibodies that Ehrlich first described as magic bullets. The philosophy of the "magic bullet" is to find the most powerful and effective molecule by synthesizing new chemicals, using as a template those already discovered. Ehrlich's serum therapy was considered the ideal approach to contend with infectious diseases—an ideal mixture of the developing medical approaches of its time.

The great chemical-pharmaceutical companies were sensitive to Ehrlich's work, first by Hoechst and later by Bayer. The researchers, now including an organic chemist, Alfred Bertheim, and a bacteriologist, Sahashiro Hata, broadened the targeted microorganisms to include spirochetes, which had recently been identified as the cause of syphilis. Some spirochetes are well-known to cause disease, including syphilis and Lyme disease. Spirochetes are long and slender unusual bacteria with axial filaments, which run along the outside of the bacterial protoplasma, but inside the outer sheath. Most of them contain a distinctive double-membrane (diderm). Usually, they are tightly coiled, and their form looks like miniature springs or telephone cords. Members of this group are also unusual among bacteria for the arrangement of axial filaments, which are otherwise similar to bacterial flagella. These filaments run along the outside of the protoplasm, but inside an outer sheath; they enable the bacterium to move by rotating in place.

Beginning the study with an arsenic compound, named atoxyl, 3 years and 300 syntheses later (an amazing large number at that time), the Ehrlich's team discovered in 1909 the drug Salvarsan (Riethmiller, 2005). Salvarsan was first tried on rabbits that had been infected with syphilis and then on patients experiencing the dementia associated with the final stages of the disease. Astonishingly, several of these "terminal" patients recovered after treatment. More testing revealed that Salvarsan was actually more successful if administered during the early stages of the disease. Salvarsan and Neosalvarsan, introduced in 1912, retained their role as the most effective drugs for treating syphilis until the advent of antibiotics in the 1940s.

Strange relations between insect-borne diseases and some pathologies

We have seen the relation between *Plasmodium* and our red blood cells, and now we are ready to join together genetic, dyes, and cartoons with insect-borne diseases. You may know of the strange cartoon creatures named Smurfs (French: *Les Schtroumpfs*; Dutch: *De Smurfen*; Italian: *Puffi*), which in the 1980s were popular in many different countries and languages, appreciated by children and their parents. They form a fictional colony of small, blue, human-like creatures. They are very nice and amusing little creatures, but something in their living style is quite strange. They live in mushroom-shaped houses in the forest with the psychedelic fungus fly agaric (*Amanita muscaria*) all around. They are virtually all males; there is just one female. The Smurfs are a population genetically separated without any outside sexual genetic breeding and this is an explanation for their unique color, which however is not a unique case. In the Appalachian mountains (USA), there is a human community quite separated from the rest of the population, and sometimes a baby comes into the word colored blue, just like the Smurfs (Trost, 1982). Similar cases were observed in other populations subjected to a limited hereditary genetic condition, such as the classic case of "Blue Fugates" or Blue People of Kentucky. However, if treated with blue methylene, the blue disappears rapidly and completely. The strange case is related to a genetic shift inside red blood cells, named methemoglobin (Fox, 1982). Methemoglobin is a form of hemoglobin, where the central ferric (Fe^{3+}) form of iron is substituted by the ferrous form (Fe^{2+}), impairing the affinity for oxygen of ferric iron and affecting hemoglobin functionality (Fig. 5.9). The binding of oxygen to methemoglobin is increased and the hem site to release oxygen is overall decreased. This is a disadvantage considering that hemoglobin is involved in the oxygen transportation method; therefore, when methemoglobin concentration is elevated in red blood cells, tissue hypoxia may occur. However, the changes in heme are once again profitable in the case of insect-borne disease, confounding the parasite. Methemoglobinemia affects about 400 million people globally. In 2015, it is believed to have resulted in 33,000 deaths and it is more present in certain parts of Africa, Asia, the Mediterranean, and the Middle East..

Another hemolytic syndrome, favism, can be treated with methylene blue (and quinine) and is related to malaria. Favism is a hemolytic disease affecting some people in particular, and it can arise from ingestion of certain pharmaceutical toxins, or broad beans (*Vicia fava*). The term "favism" is used to indicate a severe reaction occurring on ingestion of foodstuffs consisting of or containing broad beans. Most of the time, people affected by favism do not know that they have a metabolic problem, derived by a glucose-6-posphate dehydrogenase (G6PDD), which is an inborne metabolic error that predisposes red blood cells to break down. G6PDD is an enzyme contained in red blood cells and therefore carriers of the G6PDD genetic trait may be partially protected against malaria.

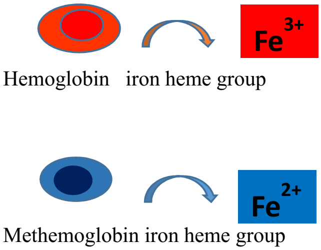


Fig. 5.9 The ionic change responsible for methemoglobin.

The symptoms, expressed within 6–24 h of the fava bean meal, consist of prostration, pallor, jaundice, and dark urine. These are the result of (sometimes massive) destruction of red cells (acute hemolytic anemia). The explanation is that fava beans are unique among other beans containing high concentrations of two glucosides, vicine and divicine, and their respective aglycones, convicine and isouramil. These compounds are powerful triggers of oxidative stress and are responsible for the characteristic hemolytic attacks.

Favism only occurs in people who have inherited the G6PD deficiency. However, awareness of the symptoms associated with favism was well-established long ago by experience, before the underlying mechanisms were understood with G6PD deficiency. The earliest reports are from Pythagoras forbidding his students to eat fava beans, and according to Diogene Laertius, even Pythagoras' death was caused by fava beans, when he was not able to cross a fava field, and in this way he was killed by his enemy. The strong aversion against Leguminosae by a rational mind, like that of Pythagoras, means that favism had already been recognized at that time as a dangerous disease with acute renal failure. This now confirmed by the common occurrence of G6PD deficiency in Greece.

Favism is more common and more life-threatening in children (usually boys) than in adults. Favism can be largely prevented by screening for G6PD deficiency and by education through the mass media or simply avoiding contact. For this reason, in supermarkets there is often advice concerning the presence and selling of fava beans. In a person who is G6PD deficient, favism can recur whenever fava beans are eaten, although whether this happens or not is greatly influenced by the amount of beans ingested and probably by many other factors. Once the attack is over, a full recovery is usually made.

It is 2000 years in Italy since we used to say "*repetita juvant*" (lit. "repeated things help" or "repeating does good"). Nobody speaks Latin any more (except in some cases in the Vatican), but some phrases are so true that they remain as roots to resist modernity asking for continuous changes. Some people affected by favism never have symptoms, and males are subjected to the disease more often than females. The reason for this difference is that favism is an X-linked recessive disorder, meaning that the responsible allele is located in chromosome X. Males possess only one X chromosome, inherited via the mother, and if the chromosome contains the gene that results in a defective G6PDD enzyme, the disease will be developed. In contrast, the presence of two X chromosomes in females allows a compensation that could mean they avoid the disease. The phenomenon is called X-linked recessive inheritance, meaning that the gene causing the trait or the disorder is located on the X chromosome. A mutation in a gene on the X chromosome causes the phenotype to be expressed in males (who are necessarily hemizygous for the gene mutation because they have one X and one Y chromosome) and in females who are homozygous for the gene mutation.

Several mutations of the allele that cause G6PD deficiency are found on the long arm of the X chromosome. Among them, two variants (G6PDA and G6PD) are the most common in human populations. G6PDA has an occurrence of 10% of Africans and African-Americans, while G6PD is prevalent in the Middle East. It is known as Mediterranean, being largely limited to people of Mediterranean origin (Spaniards, Italians, Greeks, Armenians, Sephardi Jews, and other Semitic peoples). Both variants are believed to stem a defense against malaria parasites. It is particularly frequent in the Kurdish Jewish population, wherein approximately one in two males have the condition, and the same rate of females are carriers.

The final consideration is that there are genetic solutions against malaria, but so far they are connected to important physiological dysfunctions. This is a biological rule: on the extreme sides of Boltzmann's curve, nature, through biological variation, provides lateral escapes, ready to be useful in case of future necessity. The study of these genetic situations is important and can be a starting point to develop new solutions.

However, methylene blue properties still have more to astonish us. Methylene blue was found also to be a potent antioxidant and nowadays there is a strong interest for its use in antiaging cosmetics due to its positive regenerating and protective action on skin cells. Coming back to the antimalarial drugs story, using methylene blue as a prototype, in 1934 chemists of the famous pharmaceutical company Bayer in Germany were able to synthetize resochin, which was effective but also quite toxic. Among the 1600 tested synthetic compounds during the Second World War, resochin, renamed chloroquine due to the presence of chlorine in its structure and its evident similarity with quinine, turned out to be the most effective, becoming a mainstay for malaria treatment despite its side effects. Chloroquine was therefore widely utilized from that time on, becoming the leading antimalarial drug. It was the template of a series of quinolone and quinoline antimalarial drugs of primary importance (Fig. 5.10).

Several current antimalarial drugs are utilized for the preventive treatment of malaria, named as prophylaxes, consisting of the ingestion of the appropriate dose before travel to the country where malaria is endemic.

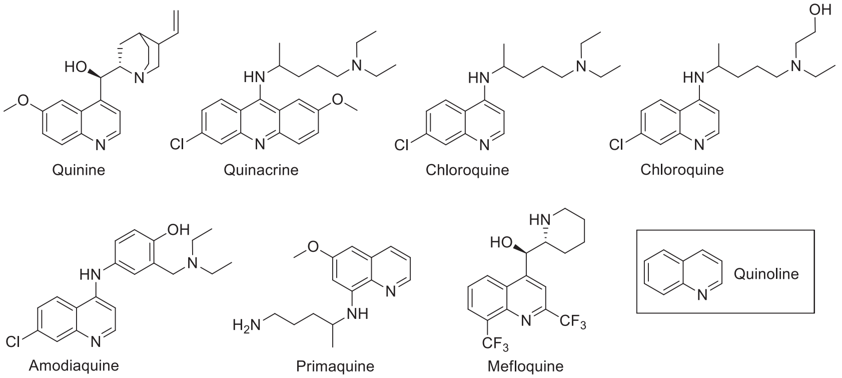
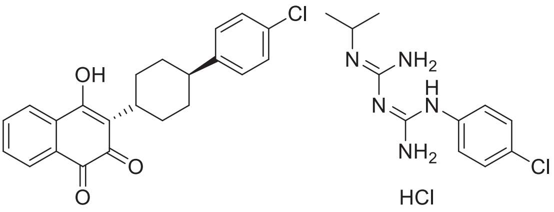
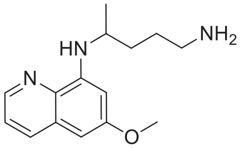


Fig. 5.10 Structures of chloroquine and related quinolone antimalarial drugs.



Malarone

Fig. 5.11 Structure of malarone (right) usually utilized with atavaqone (left).



Primaquine

Fig. 5.12 Structure of primaquine.

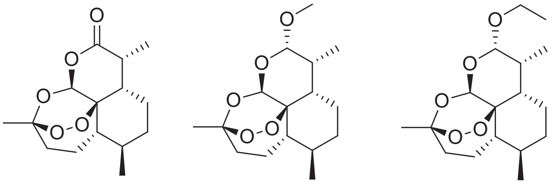
Malarone (Fig. 5.11) merits a special mention, used alone or in association with other molecules, such as primaquine (Fig. 5.12) and mefluoquine. Malarone presents an ortho-quinone structure completed by an enol functional group, which can also be converted to chetone by a cheto-enolic conversion.

Ten years later, another war broke out, again in the East Orient: the Vietnam War. The North Vietnamese army was ravaged not only by the

USA military force, but also by malaria. Because soldiers were dying from this, effective antimalarial drugs were needed immediately on the battlefield. Again, quinine was difficult to find. With China being allied to North Vietnam, although not directly involved, the Chinese Chairman Mao Zedong ordered research about herbal remedies used in Traditional Chinese Medicine (TCM) against malaria. TCM is a treasure trove of medical indications, carefully described in many documents. The remedies of TCM need scientific validation, but they are considered a reliable starting basis for the production of pharmacological and medical drugs.

Many of them were utilized only because of the bitter taste, usually considered in antipyretic effects, but few contain active antimalarial constituents. The resulting list of plants was enormous, but among the hundreds of malaria treatments, the extract of sweet wormwood (*Artemisia annua*) emerged as very efficient in reducing fever. *A. annua* is a herbal species of the family Asteraceae. The term *annua* was assigned to this species since among the 400 species of the genus *Artemisia*, it is the only annual one. The genus is well-known for the species utilized in the production of liquors, such as *A. absynthium*, utilized for the production of absinthe; the same use also concerns *A. dracunculus* and *A. genepì*. The plant presents an erect brownish or violet brown stem, well-ramified with small leaves divided by deep cuts into two or three small leaflets, which are colored in lively green and characterized by an aromatic scent. The numerous tiny yellow-green flowers are arranged in loose panicles. Although the plant is common in China and temperate Asia, it can be found also in Europe, although its presence is discontinuous. In TCM, the infusion of the leaves is mainly reported as an antifever remedy, but the treatment of malaria is clearly evidenced in a text by the Chinese author Ge Hong (341 CE) and in a document found in a tomb in 168 CE. Later, it was time for science to take center stage. In 1972, the experiments on ether extract at low pressure of the leaves fully confirmed the toxic activity against *Plasmodium* and the Chinese researcher Tu Youyou isolated artemisinin also from two other *Artemisia* (*A. apiacea* and *A. leucea*) (Tu, 2011). One year later, thanks to the scientists at the Yunnan Institute of Materia Medica (China) and Shandong Institute of Traditional Medicine and Materia Medica (China), the active crystalline ingredient was obtained and the structure determined.

The compound was named “huanghaosu” or “huanghuahaosu,” and later renamed “qinghaosu,” but universally it is known as artemisinin (Fig. 5.13). Artemisinin was immediately considered an alternative to chloroquine, due to increasing resistance to chloroquine and related drugs.



Artemisinin Artemether Arteether

Fig. 5.13 Structures of artemisinin and related compounds; note in particular the presence of the peroxide group and the number of carbons linked to two oxygens.

Animal trials on rodent malaria parasites with the crystals achieved excellent results in efficacy, toxicity, and safety, though the trials indicated problems concerning its solubility in water. In the plant extract, several other constituents help the solubilization, but the solubility changes for artemisinin, in case of its pure crystalline form, in consideration of the apolar basic structure and the presence of few active groups.

Artemisinin is a molecule completely different from quinine, chloroquine, and related compounds. The novelty is that this is not an alkaloid, completely debunking the theory that nitrogen is necessary for the activity. In fact, the bacteria do not possess a nervous system, although the neurologic side effects are of course related. Furthermore, whereas *Cinchona* is a subtropical and tropical genus, *Artemisia* is typical of temperate regions. Although most people consider the plant as Chinese, *A. annua* is commonly present in Europe, and other species of the genus are well-known for other reasons, such as *A. absinthium* for the famous liquor. The *Artemisia* genus belongs to the Asteraceae (formerly Compositae) family, wherein alkaloids are rare and terpene and phenols dominate in the secondary metabolism.

Artemisinin is a sesquiterpene lactone with unusual functionality, as tested by the presence of an extraordinary concentration of oxygenated functions, including a rare endoperoxide bridge, which is essential for its antimalarial activity. Chloroquine is mainly a flat etherocyclic molecule with a hyperconjugated center, based on two moieties. In contrast, artemisinin has a complex compact tridimensional structure full of chiral centers. However, its most interesting function, the peroxide, constitutes its Achilles heel. In a terpene, based on a hydrocarbon skeleton, the presence of oxygen is a sign of reactivity and instability at the same time. The oxygenation of the terpene skeleton must be considered a peril for the stability of the natural product, but it enhances its reactivity. Being very reactive, artemisinin is also unstable with a half-life of about 1 h, and this is a problem in treatments and precludes the use for malaria prevention. Artemisinin's discovery represented the advent of a completely new class of antimalarial agents, and, considering the success of artemisinin, several derivatives were proposed to improve its performances, like bio-disponibility and solubility in water or oil. The carbonyl group in artemisinin was reduced to obtain its derivatives, such as the water-soluble artesumate and the oil-soluble artemether in 1987, and dihydroartemisinin (DHA) in 1992. These derivatives showed a great antimalarial activity, and therefore they were introduced in the most important antimalarial drugs. However, again the natural source is easy and economic. Considering that the commercial source of artemisinin is still from the *Artemisia* plants, yields vary significantly depending on growth and habitat conditions, and various attempts to select high-producing cultivated plants or to obtain the drug using bioengineered microbes have been reported.

Considering the clear differences with quinolones, novelties were expected also regarding the mechanism of action of artemisinins. Also in this case, there are some aspects that are not clear. According to some researches, the drug acts by lowering the energetic cell source represented by the mitochondria. Metabolism of artemisinin and its derivatives is believed to be mediated primarily by the liver cytochrome P450 enzyme CYP2B6. This is in accordance with the particular oxygenated structures of artemisinins and the potentiality of carbon-centered generating free radicals or reactive oxygen species (ROS), since they are in situ activated to react.

Usually, free radical forms of oxygen are considered toxic, and several plant drugs containing phenols are used as antioxidants and antiROS. However, in this case the particular endoperoxide bridge characteristic of the trioxane pharmacophore of artemisinins is essential for their antimalarial activity. When the peroxidic oxygen is replaced with a carbon (e.g., 1-carba-10-deoxyartemisinin) the derivative is devoid of antimalarial activity. However, endoperoxides, being rich of oxygen, are dangerous for the stability of the compound. In fact, we are in presence of a molecular paradox: too activity is important to attack the parasite, but it is also a risk for the stability of the compound. This is an advanced version of the magic bullet theory, which is called by pharmacologists a *pro-drug*, meaning that it does not act in the original structure but becomes active by transformation after administration of the drug. The molecule is able to pass several passages of demolition maintaining intact its structure and only in presence of the target generate its active form. This is an aspect typical of several natural products, practically designed to obtain such results. Through this functionality, the compound should modulate parasite oxidative stress and reduce the levels of antioxidants and glutathione (GSH) in the parasite. As confirmation, artemisinins autoinduce P450 metabolizing enzymes, resulting in lower serum concentrations of the drugs in subsequent administrations. As peroxides are known sources of ROS, this finding has inspired the design of the next generation of antimalarial endoperoxides, including a number of derivatives such as the two bioactivation characters previously reported. The idea, on the basis of the synthesis of the derivatives, is join the oxygen-generating ring of artemisinins (Fig. 5.13) with the reductive scission models binding of low-valent transition irons (ferrous heme or nonheme, exogenous Fe^{2+}). The open peroxide model suggests that the ring opening of artemisinins may be driven by protonation of the peroxide or by complexation with Fe^{2+} electron transfer inducing reductive scission of the peroxide bridge to produce an O-centered radical, which self-arranges to generate a C-centered free radical.

Once formed, artemisinin-derived free radicals cause damage to cellular targets in their vicinity through alkylation. However, to obtain the oxidation of the erythrocyte membrane and consequent damage of the parasite, high concentrations of artemisinins (or chloroquine quinolones) are necessary and this is an open door for the insurgence of the resistance phenomenon. Using the membrane channels, the *Plasmodium*-resistant population of the erythrocytes is able to extrude part of the antimalarial drug, lowering its concentration to nontoxic levels. The mechanism is well-known, as experienced for several medicinal drugs, in particular in the case of anticancers. Furthermore, high doses of antimalarial drugs in the attempt to enrich the therapeutic doses increase the collateral effects. In the case of artemisinins, the limited tendency of these radicals to damage selectively cellular targets for intramolecular reactions, like typical alkyl agents, must be considered, and some data are in favor of artemisinin-derived radicals low toxicity.

A proposed mechanism concerns the classic heme hypothesis, the same already proposed in case of quinolone antimalaric drugs. Evidence supports the hypothesis that quinoline antimalarials act on the digestive vacuole of the parasite, interfering in polymerization of heme units to form hemozin by ferriprotoporphyrin IX. Quinine prevents the hemozoin crystals from growing by intercalating the quinolone rings between the aromatic groups of the ferriprotoporphyrine molecules. Hemes, being produced while breaking down hemoglobin in human red blood cells, are toxic to the parasite in high concentrations. Therefore, the mechanism of action considers that quinine binds to heme by the formation of an intramolecular hydrogen bond, interfering with hemozoin formation, thus leaving toxic ferriprotoporphyrin IX heme to thwart the parasite.

However, quinolones and artemisinins are completely different in their structures and the heme hypothesis has also been challenged. In fact, not all pharmacological data are congruent. For instance, the ability of mefloquine to affect hypnozoites is difficult to explain solely by prevention of heme formation. Mechanisms of action have been suggested, such as inhibition of vacuole-vesicle fusion or binding to essential proteins in the parasite. It was observed that during the development of parasites inside erythrocytes, the fusion of digestive vesicles occurs, giving rise to a large digestive vacuole. Instead of the mechanism preventing the heme's sequestration into hemozoin, another explanation of antimalarial drugs may be related to oxidative damage of digestive vacuole macromolecules and membranes, blocking its action. The digestive proteolysis is realized by proteases that contribute to hemoglobin breakdown, as well as other DV-associated proteins. Therefore, the digestive vacuole is important for the parasite, allowing the parasite to process 60%–80% of the erythrocyte hemoglobin. In this way, the *Plasmodium* cells can utilize the pool of amino acids, which are necessary for their survival and growth. Vacuoles act as degradative organelles, thanks to their low pH value. The membrane of the organelle works by ion pumps and transporters that maintain its low pH. The study of this organelle's functionality has been pivotal in the development of parasite resistance to several antimalarial drugs. In a similar way, the activation of artemisinins should be the result of accumulation of intraparasitic heme, which may also explain the selective toxicity of artemisinins and related trioxanes toward malaria parasites. In this regard, it is necessary to recall the deep structural differences between artemisinin and quinolone alkaloids. The gradient of pH values of the cytoplasm of red blood cells is around 7.4; instead, inside the parasite food vacuole the pH reaches 5.5, determining a further protonation of chloroquine. In the degradation process of hemin, as already described, the pH of the food vacuole plays a fundamental role. Most of the degradative enzymes are optimally active at pH 4.5–5.0, which probably allows the efficient proteolysis of hemoglobin, but also ensures the chloroquine's efficacy.

Before continuing the story of the mechanism, we must remember that chloroquine, as with other antimalarial quinolones, is an alkaloid. The word "alkaloid" derives from alkali, meaning a basic reactivity, able to change the pH of an aqueous solution in favor of oxydrilions (OH^-) against hydrogenions (H^+). The word originates from Arabic *al-qaly*, meaning "ashes from the saltwort," the ash that is obtained from plants living in the sands of salted waters, like Salicornia. True alkalis are the basis of the elements of I and II group of the Periodic Table, such as K, Na, and Ca, which on reacting with water give K(OH), Na(OH), $Ca(OH)_2$, etc., whose reaction produces directly OH^-. In organic compounds, the presence of these metallic elements in great quantities is forbidden, and basicity is obtained by a different reaction. The presence of N linked to carbon(s) inside the molecule allows the reaction of the lone pair with hydrogenions, which are therefore subtracted to the aqueous solution in favor of the OH^-. However, the basicity of such azotated compounds is not efficient like the inorganic ones, therefore they are called alkaloids, similar to humans and humanoids. Furthermore, the basicity is strongly influenced by the availability of the two electrons of the lone pair, which is a consequence of the remaining part of the molecule.

Chloroquine (Fig. 5.14) has three N, at neutral pH of the N of the side chain, with a free lone pair; it is easily protonated (CQ+), whereas at acid pH even the N integrated in the quinolone ring is protonated (CQ++). This change affects the membrane permeability of chloroquine and related compounds. The alkaloid chloroquine is a lipophilic weak base, and it is able to pass through biological membranes in the uncharged form (Fig. 5.15). Once inside the vacuole acidic compartment, chloroquine is protonated and

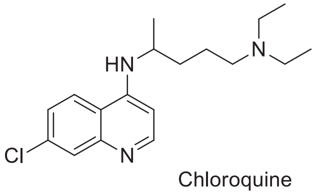


Fig. 5.14 Structure of chloroquine.

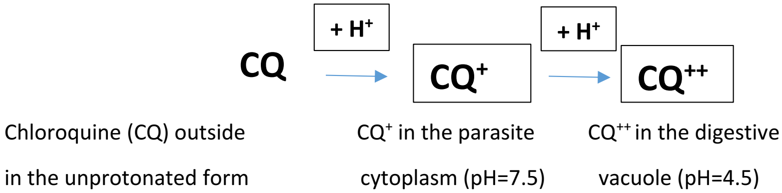


Fig. 5.15 The change of pH between the cytoplasma and the vacuole of the erythrocyte generate a double protonation of chloroquine and consequent trapping of the alkaloid.

trapped because the protonated base is relatively impermeable. The concentrative uptake of the drug means that the acid proteases of the parasite no longer function effectively. However, the parasite actives respond to restore the extrusion of chloroquine by the mechanism that we call Erythrocite membrane resistance.

The parasiticidal effects of artemisinins are effective on early ring-stage malaria parasites with little hemozoin, as well as observed in *Babesia* and *Toxoplasma* parasites that do not form hemozoin. Other models may be compatible on the basis of iron-dependent generation of ROS, calling for a role of the C- or O-centered radicals with the cellular redox systems or lipids. The explanation of the antimalarial activity is suggestive, being related to the peroxide bridge typical of artemisinins structures.

In this case, the high energy forms of oxygen, so far considered dangerous and negative, could exert positive effects in situ, revealing a reverse of the dominant axiom, but this is perfectly coherent with the Greek ancient term of a drug, *pharmakon*, meaning both medicine and poison. The meaning of this paradox is that something devoid of physiological effect is useless, but too much activity must be carefully utilized.

The future

The results of the fight against malaria are fluctuating. After a period of great success against the disease, by the global spread of DDT and antibiotic availability, malaria restarted when this pesticide was prohibited due to its environmental damage. The wide use of chloroquine has also been crucial in limiting malaria incidence, but over time resulted in high levels of drug resistance by the malaria parasite *Plasmodium*, particularly the most prevalent type, *P. falciparum*. Resistance increased every year, and nowadays it is considered that more than 50% of this parasite infecting people is resistant to treatment with chloroquine. The potential development of resistance to artemisinins by *P. falciparum* is a subject of close surveillance and extensive research. Studies at the Thai-Cambodian border, a historical epicenter of multidrug resistance, raised considerable concerns on resistance development, due to reduced susceptibility to artemisinins by prolonged parasite-clearance times. The future scenario is that all available antimalarial drugs will become ineffective, with the partial exception of quinine treatment, which has much less parasitic resistance around the world. However, quinine-resistant strains of *P. falciparum* were also reported, although in very limited numbers compared to other antimalarial drugs.

Although global incidence is still at high levels, in recent years malaria infection rates have been falling worldwide, with special reference to Sub-Saharan Africa, thanks to a general approach involving an increase of hygiene, environmental control, nutritional care, and other factors, in addition to drug treatment. However, several issues could reverse this positive tendency. Besides the already named resistance, other social factors such as human migration, social and political instability, shortage of food and other supplies, together with exponential increase of population must be considered. A number of malaria prevention and control tools currently available in advanced countries are becoming prohibitively expensive, and are thus not readily available for poor and marginalized populations in tropical and sub-tropical areas worldwide.

In conclusion, as in 2015, new chapters were added to the secular struggle against malaria, such as the Nobel Prize assigned to Y. Tu for the discovery of artemisinin and some advances in vaccine development. This was a recognition of the importance of the research against malaria and the need to explore new drugs, including as a priority natural products.

In any case, the number of people affected by malaria is still very high and there is a real need for new approaches to boost malaria prevention and control. On the drug side, after chloroquine, parasite strains resistant even to artemisinin have been detected, with quinine still remaining the only effective, but not decisive product. Furthermore, the RTS,S vaccine does not offer protection against *Plasmodium vivax* malaria, which predominates in many countries outside of Africa. Recently, a massive campaign of vaccination was undertaken in Mozambique, including also neighboring countries, and this will be an important test of the efficacy of this approach.

The fight against malaria

In summary, the malaria story could be at a sensitive point. Throughout history, it was possible to rely on several, but limited in number, efficient antimalarial medicines as powerful tools in malaria control. Drugs using *Plasmodium* as their target have developed different modes of action, developed to find the weak point of the microbe. Several successes were obtained, but only partially. Over the past 10 years, there has been a substantial regress of the enormous burden of malaria globally using a strategy involving several approaches in treatment of the disease: (a) improvement of living conditions and hygiene, including possible prevention measures; (b) introduction of new strategies of control, like insecticide-treated bed nets; (c) improved access to early diagnosis; (d) introduction of drugs combination therapy; (e) limitation of reproduction and diffusion of the vector; and (f) production and validation of vaccines.

The financial support for specific current programs on malaria highlights the production of effective vaccines, new antimalarial agents, and other novel control interventions. However, the key current challenge concerns the resistance phenomenon, based on the spread of parasite strains that are resistant to multiple antimalarial drugs. If this challenge is lost, the scenario of an exponential increase in morbidity and mortality in many malaria-endemic countries could be expected. After the development of resistance to medical drugs from the chloroquine family, artemisinin was considered a significant new hope. The key of any medical drug, even if positively validated for centuries in the perfect tests in thousands of human subjects, exactly as in traditional medicine, is the elucidation of the mechanism of action. The definition of the action of a drug is not only the necessary tool against empiricism, but i is indispensable to improve the medicament and avoid toxicity and side effects. However, it is not easy. In the knowledge of the dimension and characters of the concentric and interactive worlds, that still are not sufficiently explored, the most important one consists in the molecular level. It is also very difficult for us to understand. Nobody was able to see a molecule, or even indirectly perceive its presence. We need to utilize complicated devices to catch molecules and only after a complicated process of separation. It is necessary to imagine the pathway of the molecule after the entrance in our body, consisting in a myriad of dangerous and misleading molecular meetings to enter finally the right place and interact with the exact molecular target. It is like finding a needle in a haystack. However, it does work and is an irresistible challenge for human nature, probably the most important, as it is related to health. In this case, we have to face the multiform organism *Plasmodium*. There may be a lot to investigate about the mechanism of action of antimalarial drugs and consequent resistance.

Although reported cases of malaria are in decline and most of them evidence a static endemic situation, the disease cannot be underestimated. Firstly, although the general aspects are as described, the toxicity is highly variable, since the vector and parasite can be different. One percent of symptomatic infections may become complicated and develop into severe malaria. Severe malaria cases may manifest as anemia, hypoglycemia, metabolic acidosis, repeated seizures, and/or multiple organ failure. Other possible symptoms are jaundice, shock, renal failure, lactic acidosis, abnormal bleeding, pulmonary edema, and adult respiratory distress syndrome.

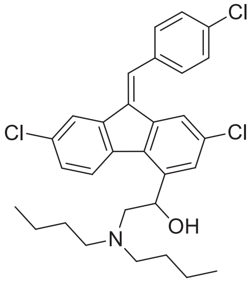
Cortical infarcts and cerebral venous or dural sinus thrombosis as part of disordered coagulation may also develop. Bacterial coinfection may be observed, particularly in those with shock, and accounts for the majority of late deaths, but the most delicate target is the CNS.

Malaria is still a huge threat for public health and socioeconomic development in resource-limited settings of the world. Data appear positive. Malaria mortality rates have fallen by 47% globally since 2000, and by 54% in Africa. Most deaths occur among children living in Africa, where a child dies every minute from malaria, but this is also decreasing. However, the trend could be reversed due to the resistance phenomenon.

To deal with the serious situation caused by resistance, scientists are exploring new strategies, first repudiating the pharmaceutical axiom based on a single active molecule, embracing the philosophy of the multicomponent/multitarget. In the case of artemisins, artemisinin-based combinatory therapies (ACTs) have been introduced and widely deployed in malaria-prone regions. An example is the utilization of armethere in combination with lumefantrine. From the comparison of the structures of the two compounds, we can argue that whereas armethere is a sesquiterpene derived from artemisinin, lumefantine (also known for its use as coarthemeter) is a very different compound (Fig. 5.16). Therefore, there is a mixture of antimalarials of first and second generation.

Current and future research strategies to eradicate malaria can follow different approaches, including the following:

(i) The classic pharmacological treatment based on the active molecule that, according to the "magic bullet" paradigm. This "magic bullet" is a chemically defined molecule with a pharmacologically known mechanism and target, the activity of which is confirmed by clinical trials. It can be of synthetic or natural origin, albeit the former is usually



Lumefantrine (benflumetol or co-artemether)

Fig. 5.16 Structure of lumefantrine.

preferred by pharmaceutical industries. So far this approach, as for other diseases, is inevitably leading to resistance by the parasite.

(ii) The use of natural products, like quinine and artemisinin, as templates for production of a range of active molecules. The possibility of finding useful natural products is high, but there is no valid strategy to select the right molecule. So far, results have been obtained mainly using ethnopharmacology indications, but this requires careful scientific validation and could need tremendous screening.

(iii) The development of a vaccine, announced several times and so far still in progress. The vaccine hypothesis seems quite difficult, and so far insect-borne diseases have not been totally solved by vaccines.

(iv) Searching, or waiting, for natural mutations of the parasite into less aggressive forms, making the host tolerant. This involves more or less just hope, and the target is still argued, in terms of whether to try to change the parasite and/or the vector.

(v) The control of mosquito vectors, mainly based on insecticidal treatments against mosquito larvae, with serious impacts on the environment and human health.

So far, no single strategy can be considered preferable, and the mixing of more strategies is considered prohibitively expensive.

Exploring the future, some other considerations are necessary. Endemism is a typical characteristic of malaria. Thus, despite eradication in the developed parts of North America and Europe, if you are born where malaria has been endemic for at least hundreds of years, there are two possibilities: you will be a victim of the disease, like most patients 5 years or younger, or you will learn to live with the disease for the rest of your life.

Another consideration is also necessary, though it can be considered apathetic, since it is not completely respectful of the human life. The number of people regularly killed by the insect-borne diseases is so relevant that in case of sudden absence of such mortality, the quantity of new survivals will change drastically the number of inhabitants for several countries, causing enormous problems about the resources of water and food, with enormous pressure and instability. The inherent cause is that this epidemiologic situation is the consequence of a complex interplay of multiple factors. These include the mosquito vector, like density, ambient and environmental conditions, population movements, altitude, parasitemia rates among endemic populations, and the anopheline mosquito species. Others are related to the parasites, like resistance and virulence of strains.

Viral insect-borne diseases

In Table 5.2, we can see that insect-borne diseases caused by viruses are prevalent against the bacterial ones. This could be surprising considering the extreme simplicity of viruses structure against complexity and capacity of bacteria, but evolution and functionality must not always be in accordance, as evident in the current presence of old forms of organisms. As is well-known, viruses are very different from bacteria and are not considered as living organisms, though many aspects of their reactivity are similar, as evidenced by insect-vector diseases. Usually, in case of infection, people use to seek help in the antibiotics, ignoring the fact that they are not useful against viruses, also as consequence that the pool of efficient antiviral drugs is very limited.

This part of the book is focused on a special actor, very clever and able. It is simply a molecule but one that is able to undergo the processes already encountered, and find vectors and hosts to organize the superorganism strategy. This is an example of the absence of fundamental differences of behavior between organisms and other molecular matter. The most important difference is that the virus strategy is based on patience and capacity to wait, hidden deep inside the body of the host without any evidence of its presence, until the moment it strikes. Usually, the symptoms are not evident and the disease generally does not involve the death of the host, which would be negative to the infected agent and necessary for the agent proliferation (Table 5.5).

Viruses involved in insect-borne disease are a special category, coming from a long selection. They are classified as arboviruses, consisting of a heterogeneous group of viruses transmitted by a hematophagous arthropod vector. Ticks, sandflies, and mosquitoes are their vectors, and they predominate in tropical regions. The most well-known and severe arthropod-vectored diseases are caused by an arbovirus, like yellow fever, *togaviridae*

Table 5.5 Classification of *Flavivirus.*

Unranked	Virus
Realm	*Riboviria*
Phylum	*Incertae sedis*
Family	*Flavivirus*
Type species	Yellow fever virus

(equine encephalitis) chikungunya virus, and *bunyaviridae* (California encephalitis and Bunyaviral hemorrhagic fever). However, we must assign a special place to *flaviviridae* (dengue, yellow fever, St. Louis encephalitis, and West Nile encephalitis). The difference in viruses concerning vector-borne disease consists of the behavior inside the host. Human infections with most of these arboviruses are incidental, as humans are unable to replicate the virus to high enough titers. Humans are then a dead-end host, enabled to re-infect the arthropods needed to continue the virus life cycle. Yellow fever, dengue, and Zika viruses still require mosquito vectors, but are sufficiently well adapted to humans not to depend upon animals. Viruses are not considered organisms, but the arboviruses' behavior is very similar to that described in other vector-borne diseases. We may consider this as an example of evolution without selection.

Flavivirus is a genus of viruses in the family Flaviviridae. The name is derived from yellow fever, which in turn is named for its propensity to cause yellow jaundice in victims. *Flavi* or *Fulvi* (indicating fair hair) is the historical name of a dynasty of blonde Roman emperors, as reported by several historians. Suetonius described Augustus as having "*subflavum*" hair, and "his complexion was between dark and fair" (*Augustus*, 79). Nero also had "*subflavo capillo*," in contrast to the typical Mediterranean style. Wilhelm Sieglin compiled a list of 27 blond-haired Roman gods and goddesses, 10 blond Roman heroes and heroines, and 63 blond Roman historical figures, many of whom were Patricians. This could confirm some genetic theories based on frequent use by the Roman Patricians of names such as *Rufi*, *Flavi* or *Fulvi* (indicating yellow hair), and *Caesulla* or *Ravilia* (indicating light eyes), demonstrating strongly Nordic racial affinities and genomic influences.

Essentially, a virus is a molecule of nucleic acid inside an envelope. The general structure of the cell is the same, consisting of concentric spheres. The virus core is an RNA or DNA molecule, which is the active part, consisting of only 10,000–11,000 bases (compared to our 3 million pairs of bases). The virus is enclosed in a protein coat, named a capsid. The capsid is surrounded by a glycoprotein envelope, wherein surface proteins are inserted, helping the core to perceive the environmental changes and attach to its host cell. Dimensions are ultramicroscopic (20–300 nm in diameter, much smaller than a bacterium) and therefore the virus pertains to the molecular world. Its shape can be very varied and complex (icosahedral, helical, etc.). The main difference to bacteria and other organisms based on cells is the absence of growth: viruses only exist to make more viruses. A virus is a very efficient molecular mechanism, dedicated to the multiplication of its molecule. The virus particle attaches to the host cell's external envelope before penetrating it. The virus then uses the host cell's machinery to replicate its own genetic material, obtaining a copy of its genome. As the newly formed viral particle pushes against the host cell's plasma membrane, a portion adheres to it. The plasma membrane reacts and envelops the virus, but the cell membrane becomes the viral envelope. The virus core is then released into the cell. This process slowly uses up the host's cell membrane and metabolism, and usually leads to cell death. The virus particles burst out of the host cell into the extra-cellular space, resulting in the death of the host cell. Once the virus has escaped from the host cell, it is ready to enter a new cell and multiply, causing an increase of the infection. Viruses are not able to obtain replication alone, since they are metabolically inert and therefore all viruses are obliged to be infectious agents.

Therefore, in conclusion there are no good viruses, since they replicate only within the cells of living hosts, causing cell damage. Once replication has been completed, the virus particles leave the host by either budding or bursting out of the cell (lysis).Viruses attack any form of living organisms, including bacteria, and their subunits, like white blood cells.

The idea considering the viral insect-borne diseases limited by the simplicity of viral structure, mainly consisting of a nucleic acid material, can be misunderstood. This molecule is able to perform several activities that are considered peculiar to living organisms. Viruses in *Flavivirus* are linear positive-sense RNA enveloped in a capsule with icosahedral and spherical geometries. There are 32 Flavivirus species recorded as virulent for mankind, including West Nile virus, dengue virus, tick-borne encephalitis virus, yellow fever virus, Zika virus, and several other viruses which may cause encephalitis. They are classified as arboviruses, since transmission is a consequence of the bite from an infected arthropod (mosquito or tick). The viruses can infect several hosts, with important animal transmission routes, but humans are considered dead-end hosts, being unable to replicate the virus to high enough titers to re-infect the arthropods needed to continue the virus life cycle. However, there are important exceptions in yellow fever, dengue, and Zika viruses. These three viruses still require mosquito vectors, but are sufficiently well adapted to humans not to depend upon animal hosts. Therefore, again there is concomitant participation, potentially involving transmission from nonhuman vertebrates to humans with an intermediate vector arthropod. The known nonarboviruses of the *Flavivirus* family reproduce in either arthropods or vertebrates, but not both, with one odd member of the genus affecting a nematode. As a consequence, *Flavivirus* utilizes different strategies to infect the host and replicate. In addition to the codification of viral proteins, inside the genome virus consisting in its RNA, there are special signals that are able to activate counteractions regulating amplifications of viral components. In the adaptation to the type of host, special roles have been assigned to the parts of the RNA present in the non-codifying region of the *Flavivirus*. As a confirmation, dengue virus is able to adapt to different hosts, like mosquitoes or human cells, by selection of different viral variations. The adaptation to the host is performed by viral structures present in the 3'UTR region, which is associated to improvement of the so-called "viral health." Therefore, replication of the elements of the viral RNA changes according to the different involved cellular types. In some aspects, this can be considered an adaptation, like the metamorphoses described earlier. That means that there is no absolute need for the cell envelop to obtain some results by the nucleic acid. Other virus transmission routes for arboviruses include handling infected animal carcasses, blood transfusion, childbirth, and consumption of infected food, such as unpasteurized milk products.

We can now consider the characters of each viral insect-vectored diseases, always remembering that inside any infection there is a complicated mechanism.

Dengue

Dengue is another of the most important mosquito-borne diseases in the world (Hong-Juan et al., 2012). Again, it is spread in tropical and subtropical regions, but the pathogen is an arbovirus, which is the general name assigned to viruses affecting mankind. In this part, we shall encounter arboviruses several times. Arboviruses are the main agent of several arthropod-borne diseases. The term "arbovirus" is self-explicative of this intimate relation, since the "ar" refers to arthropod, and "bo" refers to borne. In the arthropods, we include mosquitoes, ticks, fleas, and gnats. In the main classifications, arboviruses are divided into *Flavivirus*, *Alphavirus*, and *Bunyavirus*. *Flavivirus* are single-stranded, enveloped RNA viruses. These viruses are all transmitted by either ticks or mosquitoes. West Nile virus, Zika virus, and yellow fever are examples of mosquito-borne flaviviruses. *Alphavirus* also includes single-stranded RNA viruses, but they have a coated virion, which is the infective active component of the virus. *Alphavirus* is transmitted through mosquito bites and is responsible for chikungunya, Ross River virus, and both western and eastern equine encephalitis diseases. *Bunyavirus* includes enveloped RNA viruses, which can be transmitted by arthropods or rodents. We shall focus on the ones that are transmitted by arthropods, including ticks, mosquitoes, and various types of flies. The diseases attributed to *Bunyavirus* include Jamestown Canyon virus and California encephalitis.

Arboviruses are enveloped. The virus envelopes protect the inner part, which contains the capsid with the viral genome. Envelopes are chemically constituted by phospholipids and proteins, derived from cell membranes, as well as some viral glycoproteins. Thanks to the envelope, viruses try to avoid the host immune system, and glycoproteins on the surface of the envelope serve to identify and bind to receptor sites on the host's membrane. In this way, the viral envelope fuses with the host's membrane and defeats the host cell's external defenses, and the capsid and viral genome can enter, causing infection. Once inside, the virus can locate the molecular resources necessary for its replication.

Therefore, most vector-borne diseases are consequences of the ability of a virus to infect and diffuse inside a host cell. Despite this astonishing capacity, the arbovirus is a very simple molecular machine. Simplifying, we have a protein envelope and a nucleic acid core, plus some enzymes. If you want to control a virus, you must mainly target on the external barrier, the envelope. Without this protection, the virus can be easily attacked by the immunity system or a vaccine. Current researches on viremia, such as Zika or Ebola, are focused on this aspect. The idea is that when deprived of its protection, consisting in the involucre, the virus can be defeated by our natural defenses, without any other external intervention. In such approach, sustain the general health state of the patient is fundamental.

In the case of dengue, the viruses belong to the genus *Flavivirus*, whereas the main vector is *Aedes aegypti*, followed in a lesser degree by *Ae. albopictus*. The incidence of dengue, in contrast to the situation of malaria, has grown dramatically around the world in recent decades. The main difference between dengue and malaria diseases is that a virus is responsible for the infection and the illness. Other differences must also be considered, however. Dengue has emerged as a worldwide problem since the 1960s, and is reported as endemic in more than 100 countries in Africa, America, and the Eastern Mediterranean, but South-East Asia and Western Pacific are the most affected by the disease. Although mainly hot countries are impacted, including tropical and subtropical areas, the disease is common in many popular tourist destinations in the Caribbean (including Puerto Rico), Central and South America, South-East Asia, and the Pacific Islands.

In the United States, local spread of dengue occurs infrequently. Dengue is the most common and important worldwide viral disease transmitted by mosquito vectors. No vaccines are available and therefore vector control with insecticides remains the most important tool for prevention and interruption of the transmission of this disease.

Anyone who lives in or travels to an area with risk of dengue is at risk of infection. Before you travel, you should locate country-specific travel information to help you plan and pack. Dengue is considered a typical disease for passive dispersion, meaning the spread of the vectors and dengue virus from one region to another through means of transport. The increase of tourism is one cause of diffusion of vector-borne diseases, and in such cases, most of the classic considerations about insect-vectors must be totally revised.

Until 2005, the reported data considered about two-fifths of the world's population to be at risk of acquiring dengue. However, the numbers of dengue cases, as for other insect-borne diseases, must be considered only an indication, since many situations are underreported and misclassified. Reported data tell us that more than 2.5 billion people, corresponding to more than 40% of the world's population, are now at risk of dengue infection and there are 50–100 million cases of acute febrile illness yearly, including about 500,000 cases of severe dengue. The incidence of dengue has grown dramatically around the world in recent decades. According to WHO current estimations, there may be 50–100 million dengue infections per year, but this number is the medium of an estimate interval 284–528 million, of which 67–136 million are manifested clinically, considering any severity of disease, 0.5 million are hospitalizations, and 22,000 deaths occur worldwide every year. Another study estimates that 3.9 billion people, in 128 countries, are at risk of infection. However, it is necessary to remember that the risk of infection means a potentiality, and the disease could not be expressed in evident symptoms.

We know that a recovery from infection provides a lifelong immunity for the subject against that particular serotype. Cross-immunity to other serotypes after recovery is only partial and temporary. Currently, there is no specific treatment for dengue, although a vaccine is currently being developed. Its prevention and control depends solely on effective vector control measures. However, again the overuse of insecticides has generated resistance to several insecticides by the vector.

The agent

The disease is caused by a single stranded RNA virus of genus *Flavivirus*, comprised of four closely related but antigenically distinct serotypes, named DENV-1, -2, -3, and -4. Viruses are not considered living organisms, but the rules of homeostasis are completely respected. They are most efficient in reproduction, this being the unique pursued aim of these molecular machines. In fact, we must consider that organic matter, inside an organism or not, is subjected to the same general physical and chemical factors acting in the environment. Dengue is classified in different illnesses, such as undifferentiated fever, dengue fever, dengue hemorrhagic fever, and dengue shock syndrome, with various degrees of symptoms and gravity of manifestations, including acute fever, symptoms of headache, retro-orbital pain, myalgia, arthralgia, and rash. Typically, dengue illnesses begin 5–7 days after the infection, as the consequence of a blood meal from an infected mosquito. Within the vector, the virus infects the mosquito mid-gut and subsequently spreads to the salivary glands over a period of 8–12 days. Therefore, after the bite of the infected mosquito, there is an incubation period of 3–14 days before symptoms appear. After this incubation period, the virus can be transmitted to humans during subsequent probing or feeding. Viremia in human peripheral blood peaks during the early days (first 2–3 days) of acute illness and then declines sharply, after the introduction of virus targets directly on antiinfective human defenses. Human cell types, i.e., monocytes, macrophages, and dendritic cells, acting as major phagocytic cells of the innate immune system and responsible for detecting and removing invasive pathogens, are permissible for infection. These cells are also antigen-presenting cells critical for the initiation, expansion, and polarization of adaptive cellular immunity.

Dengue virus genome is coated with a host cell-derived lipid bilayer. The genome is an 11 kb-longsingle positive-sensed RNA molecule encoding 10 viral proteins: three structural proteins, capsid, membrane, envelope, and seven nonstructural proteins. The envelope proteins on the viral surface are major players for viral attachment, endocytosis, uncoating, and fusion, binding to cellular receptors and direct virus particles to the host cell's endosomal compartments. Dengue virus enters host cells through receptor-mediated endocytosis. Once inside, the viral uncoating occurs in the cellular endosomal compartments and subsequently viral RNA releases into the cytoplasm of the host cell, near the ER (endoplasmic reticulum), which is populated by ribosomes. It is worth remembering that in this reticulum, which is part of the cell endomembrane system, the proteins' synthesis is performed, and further the proteins are glycosylated in the Golgi apparatus, to be finally encapsulated in microvesicles and in this form travel inside the cytoplasm, before entering the cell metabolism. The viruses mimic this pathway. Through a mechanism termed "budding," the nucleocapsids join with the endomembranes, forming progeny of viral particles in the rough ER cisternae. The budding continues with transport of the viral particles to the Golgi apparatus, wherein the initially attached glycan is further modified by a complex process of trimming or remodeling, resulting in varying oligosaccharide structures.

Glycosylation is fundamental, since it can promote proper protein folding required for protein functioning, and in this case affects interactions between virus and receptors, and alters antigenic structures. However, the modified viral structure can be recognized by host immune cells and antibody, thus our body impacts viral replication and infectivity. This is our best defense against the virus, but immune efficiency can be decreased by several factors opening the way to viral infection. This equilibrium beside attack and defense, the balance between inside and alien forces, is the key of any insect-borne disease, indeed of any disease. Once again, mankind is the main decisive factor, leaving the others in a second role.

The fight against viruses, including dengue virus, has been underway for a long time, based on different strategies and targets. Research against dengue is exploring the aforementioned aspects of the disease. Attention is focused on those who experienced dengue asymptomatic infections, to address protective immunity better. Another approach includes viral and host cellular molecules involved in receptor interaction and infection. Another front is focused on the abovementioned role of glycosylation with respect to receptor binding, viral tropism, and virulence of infectivity, and enhancement of innate immune mechanism, such as adaptive humoral and cellular immunity. However, so far several aspects of the dynamic process of the different stages of dengue virus infection are not well understood.

The vector

Ae. aegypti is the primary vector species responsible for transmitting the viruses that cause dengue, but also for chikungunya fever, urban yellow fever and other important diseases involving viruses. *Ae. aegypti* has white bands on its legs and spots on its abdomen and thorax. The viruses are passed on to humans through the bites of an infective female *Aedes* mosquito, which mainly acquires the virus while feeding on the blood of an infected person, but other possibilities can occur. Mosquitoes do not naturally carry viruses—we must remember that mosquitoes must acquire them from an infected person before they can transmit them to another person. As was reported for malaria, only female mosquitoes bite, in search of blood for egg production, and therefore only female mosquitoes are responsible for transmission of the above virus diseases. *Ae. aegypti* females are usually not good fliers, since they may spend their lifetime in or around the houses where they emerge as adults and they usually fly an average of 400 m. However, several forms of passive transportation are possible and this means that people, rather than mosquitoes, rapidly move the virus within and between communities and places.

Again and as always, we must consider the human role. The life cycle of the vector may be as short as 10 days or, in cool weather, as long as several months. Therefore, the mosquitoes must survive during winter, finding refuge in our warmed houses or other protected places. The indoor habitat is less susceptible to climatic variations and increases the mosquitoes' longevity.

Ae. aegypti (as evidenced by the name) originated from Africa but is now distributed globally in tropical and subtropical regions. Distribution in new areas was assisted by mass human forced migrations, like that to the New World associated with the massive slave trade between the 15th and 19th centuries from Africa to the Americas, and then to Asia as a result of trade during the 18th and 19th centuries. A further worldwide redistribution occurred as a consequence of the Second World War following troop movements. Therefore, once again the virulence and impact of an insect-borne disease is strictly linked to human activities. Furthermore, as for other insect-borne diseases, recently rapid human population growth and increased urbanization has led to an increase of incidence, which is favored in crowded and dense human habitats. Permanence and diffusion are helped by substandard housing, inadequate hygiene conditions, insufficient and/or polluted water supply, and inadequate or absent waste management. All these factors may cause an abundance of mosquito breeding sites, but we can add storage of drinking water and other urban water containers, including plant/flower-pot bases, rain pools, guttering, tires, and small quantities of still water. Any water reservoir provides an ideal habitat for reproduction and growth of *Ae. aegypti* larvae. As for any other mosquito, the immature stages need water-filled habitats, which can consist of artificial containers closely associated with human dwellings and often indoors. This means a large spread of the vectors and their control needs the collaboration of inhabitants, since usual massive treatment of areas by insecticides can be insufficient or inefficient.

Public enemy No. 1

It is evident that *Aedes* mosquitoes are favored in human settlements, being highly adapted to rural and urbanized areas, since they generally live indoors and near people. Inside houses, mosquitoes use to rest hidden in cool shaded places, such as in wardrobes, laundry areas, and under furniture. Therefore, if during the winter we heard the typical sound of the mosquito's flight, we should not consider this presence unusual.

If you ask people about the most aggressive and dangerous creature on the planet, they might suggest a lion, tiger, crocodile, or shark, or similarly aggressive and powerful creatures. I would reply—after mankind—Aedes albopictus. All the other creatures are relatively very rare and avoid contact with harmful human beings. I remember the episode of a Ph.D. visiting student of my laboratory coming from Central Africa, who went to the Zoological Park to see elephants and lions for the first time.

This is the final act of a long history. Where are the immense mammoths and the ferocious saber-toothed tigers? They were exterminated by the inexorable traps and poisoned arrows of our ancestors. In contrast, insect vectors of diseases are still everywhere, and some of them dominate several habitats. To win the struggle of survival, you do not need force and superpowers, but smartness and perfect knowledge of your habitat, including the strategies of natural enemies.

As already reported, also in developed countries, malaria and other diseases cannot be considered ended and peril could enter from abroad, as evident in the case of *Ae. albopictus* (syn. *Stegomyia albopicta*) of the Culicidae family. Among Culicidae, this mosquito is acknowledged as the most invasive mosquito species in the world. However, in terms of its ecological and physiological plasticity, its potentiality is much higher. Its environmental impact is expanding in several countries, sustained by climate changes and concentration of human population in huge cities. *Ae. albopictus* is a dangerous vector due to its aggressive daytime human-biting behavior and its ability to transmit many viruses, including dengue, yellow fever, West Nile, and chikungunya. It may also act as a vector of filariasis, with special reference to *Dirofilaria immitis*, *Dirofilaria repens*, and *Setaria labiatopapillosa*.

In comparison with other insect vectors, even in the same genus like *Ae. aegypti*, *Ae. albopictus* has evidenced a higher migration and adaptation capacity. In recent decades, *Ae. albopictus* has spread from Asia to areas of Africa, Europe, and the Americas. Therefore, although both have been implicated in large outbreaks of chikungunya and dengue, whereas *Ae. aegypti* is confined within the tropics and sub-tropics, recently *Ae. albopictus* became established also in temperate and even cold temperate regions, expanding its distribution as soon as climate changes favored its presence. Another important characteristic is the possibility to survive and reproduce in a large range of aquatic microhabitats, rural or artificial, in peri-urban and urban areas, from shady city parks to flower vases, water storage vessels, and concrete water tanks in bathrooms—practically everywhere humans live. This mosquito has demonstrated a special capacity of adaptation, as well as the possibility to feed on many targets and therefore be an efficient vector of several viral diseases.

Depending on the mosquito's species and age, bites can be relatively painless, so persons may not notice they are being bitten or may think they are being bitten by other biting insects, or bites may be very painful and irritating for several days. Usual targets are people's feet and ankles, which mosquitoes may bite repeatedly. Another recent novelty of this mosquito is biting during daylight hours. Biting activity is higher in the 2 h after sunrise or before sunset, but it will bite throughout the day, differently from mosquitoes endogenous of temperate zones. As a result, bed nets do not prevent disease transmission. These mosquitoes are hard to catch and move very quickly, darting back and forth.

This mosquito is commonly known as the (Asian) tiger mosquito or forest mosquito, being characterized by the evident and recognizable white bands on its legs and body, which resemble those of a tiger, but also to express its violence (Roiz, 2001). Males are very similar to females, but much smaller, and their antennas are clearly thicker. Again, this species is native to the tropical and subtropical areas of South-East Asia, and it was confined for a long time in these regions. In the past few decades, it has spread to many other countries thanks to climate changes, but again mankind should take central responsibility. This mosquito was probably introduced through the transport of goods in international travel. From Asia, the tiger mosquito passed to the eastern states of the USA and then in 1990–1991 to Italy, thanks to the resistance of its eggs, and later to other parts of Europe. Italian people did not have previous experience with this mosquito, but immediately perceived its arrival. The striped appearance of this species was perceived not only for the painful bites, generating a severe itch and large swellings, but also for the all-day disturbance, with no break. In Europe and the USA, *Ae. albopictus* is an alien species in a condition to dominate its habitat. Usually the dimensions of females are superior to those of autochthonous species. The new vector was rapidly able to become a significant pest, thanks to some additional characters respect to those of native mosquitoes, which are not able to deal with the invasion. Autochthonous mosquitoes are common in water pools of any type and shape, whereas *Ae. albopictus* needs smaller pools, preferably shaded, like those easily found in human sites, especially in towns. Another important difference is in its movement capacity. Common mosquitoes often move for a few hundred meters, while other species can reach more than 10 km. The tiger mosquito lives in a limited area (*c*.200 m) and therefore egg deposition sites can be found near where the insect can be observed. In addition to humans, this mosquito also bites other mammals and birds. The female mosquito uses to integrate the bool feeding with other source of food, like nectar and other plant-produced substances, of course the same behavior of males and many other insects. During the feeding, the female is particularly perseverant and careful. In a first step, it flies around looking for the best target. Once this is decided, it starts to feed but soon suspends the feeding, before the ingestion of the necessary quantity for the development of the eggs. From any bite, usually only 2 μL are assumed, but the bite is sufficient to transfer the parasites. The mosquito visits several targets, causing the infection of many hosts, and the transfer of the disease from species to species.

The tiger mosquito is an epidemiologically important vector for the transmission of many relevant viral parasites, including yellow fever virus, dengue fever, chikungunya fever, and several filarial nematodes such as Dirofilariasis immitis. Furthermore, *Ae. albopictus* is capable of hosting the Zika virus and is considered a potential vector for transmission among humans (Romi, 1995; O'Meara et al., 1995). In particular, in Italy the alert was immediately raised after the first reports about the mosquito's presence, followed by several programs to control the diffusion, increasing every year. In Italy, the mosquito's successful integration is considered a consequence of recent climatic changes, in particular the higher temperatures in winter.

As already reported, *Ae. albopictus* is native to tropical and subtropical regions with warm and humid climate; however, it has been adapting successfully to temperate regions, where it is now able to hibernate over winter (Roiz, 2001). The history of the Asian tiger mosquito is strange, since its origins are humble, and very different from the current widespread diffusion. It originally lived on the edges of forests in East and South-East Asia, breeding in tree holes and other natural reservoirs. Centuries ago, it spread to Madagascar and Indian Ocean islands, but only in the past 50 years has it spread to all inhabited continents, and this recent diffusion is considered in large part as a result of increased global air travel and seaborne trade. The worldwide trade in secondhand tires, probably from Georgia (USA), which often contain water and are an ideal place for resistant eggs, is considered the key factor of the arrival of *Ae. albopictus* in Europe. The eggs can survive in such a refuge until the tires reach their destination. The eggs can also survive cold winters because they go into a state of dormancy or "diapause," allowing the *Ae. albopictus* mosquito to persist in areas with a temperate climate. In newly infested areas, the mosquito has adapted easily to human settlements, where pots, vases, and buckets can act as breeding sites, provided that there is a bit of vegetation. Therefore, the case of the tiger mosquito can be considered emblematic of climatic change and human influences.

Although eggs from strains in temperate zones should be more tolerant to the cold than those from warmer regions, the distribution of this species was limited since the larvae and eggs of *Aedes* sp. transmitting mosquito-borne disease cannot survive snow and temperatures under freezing. Generally winter had been the most important front line blocking the tiger mosquito's invasion. Changing these conditions, the distribution areal can consequently move to northern territories. The climate change models indicate that by 2030, the temperatures will have risen sufficiently to enable *Anopheles* mosquitoes to conquer Europe until Wales, where the climate in becoming increasingly mild and wet (Bendict et al., 2007; Caminade et al., 2012). Tiger mosquitoes are also favored because they can survive throughout winter in suitable microhabitats, and typically fly and feed in daylight. In fact, in 2018 these mosquitoes were reported in the southern and central regions of France, and consequent alerts reported 333 cases of dengue, 12 of chikungunya, and 21 of Zika diseases. These are very low numbers in comparison with the overall population, but enough to trigger concern.

The argument is why has *Ae. albopictus* so far been absent in Europe when it is now so abundant and prominent? Again, we must consider the human influence. Beside the climate changes, in particular the rise of temperatures, especially in winter, which allowed survival during the year, the mosquito adapted perfectly to urban communities being associated with inhabitants' lifestyles in major cities, whereas *Anopheles* sp. and other ordinary mosquitoes preferred living in wetlands. Furthermore, it is more aggressive and virulent in comparison with other mosquitoes, affecting the environment equilibria in favor of its needs. The danger of introducing alien species, such as *Ae. albopictus*, is a current problem of high importance, as evident in the controls at airports, and it will be necessary to return to this aspect.

Aedes larvae and pupae are usually targeted using organophosphates and insect growth regulators. Indoors residual spraying and insecticide-treated bed nets are also employed to reduce transmission of malaria in tropical countries. However, these synthetic chemicals have strong negative effects on human health and the environment, and induce resistance in a number of mosquito species. The main problem concerns the spread of the mosquito in microhabitats inside villages and towns. Therefore, eradication is difficult and ordinary methods of disinfestation are inefficient without the active collaboration of the population. Capillary information about movements and activities of inhabitants are essential in the eradication campaigns. In this case, the computer simulation of flows, obtained by monitoring of the cellular activity, are useful and the data can be crossed with project like the Gridded Population of the World by NASA checking the geographic position of each individual in a selected area. Otherwise, after the insecticide treatment, the mosquitoes can re-emerge more active and dangerous than before.

The future of dengue is not predictable, but as with other vector-borne disease, it is re-emerging in areas that have been disease-free for relatively long periods of time. The episodes are so far limited and short-lived, but some in particular are interesting. Xiao et al. (2016) reported on a Dengue outbreak in a city in southern China that had been disease-free for more than two decades. According to the details of the report, the infection, due to Dengue serotype 1, was introduced by a traveler from South-East Asia and transmitted by *Ae. albopictus*. However, *Ae. aegypti* is the most important vector of dengue, since *Ae. albopictus* is a less competent vector of arboviruses, and causes milder epidemics. Therefore, the replacement of *Ae. aegypti* with the tiger mosquito could even result in public health benefits. However, there is no solid evidence for this, and the milder course of the outbreak could in part be explained by the relatively short duration of the hot season in some affected areas. *Ae. albopictus* is rapidly increasing its changing global distribution, and its expansion could create new opportunities for viruses to circulate in new areas, becoming a common cause of epidemics in countries so far fre of *Ae. egypti*. In particular, the worldwide trade in secondhand tires or other goods, which often contain water and are an ideal place for eggs and larvae, has been a key factor in the large-scale conquest of *Ae. albopictus*, which adapts easily to new environments, even in temperate climates. Similar episodes have been described in Mauritius and Hawaii. Therefore, airport controls are important and necessary, but it is unlikely that they will block the tiger mosquito.

Chikungunya fever

Chikungunya virus (CHIKV) is an alphavirus of the Togaviridae family vectored by *Aedes* sp. mosquitoes. Perhaps due to its difficult name, this is less famous among the diseases here considered, but this is not a reason to underestimate its value and importance. We shall consider epidemiology, biology, treatment, and vaccination strategies of CHIKV, which is a case study of insect-borne diseases.

"Chikungunya" is Maconde (a local Tanzanian language) for "he who bends" and describes the huddled-up body posture caused by the chronic and incapacitating arthralgia that normally accompanies acute infection in human populations. Arthralgia (from Greek *arthro-*, joint + − *algos*, pain) literally means joint pain. The term "arthralgia" should only be used when the condition is noninflammatory, and the term "arthritis" should be used when the condition is inflammatory. Since 1952, CHIKV has been responsible for sporadic and infrequent outbreaks. However, since 2005, global CHIKV outbreaks have occurred, inducing some fatalities and associated with severe and chronic morbidity. CHIKV is thus considered as an important re-emerging public health problem in both tropical and temperate countries, where the distribution of the *Aedes* mosquito vectors continues to expand. The virus is endemic to tropical regions, but the spread of *Ae. albopictus* into Europe and the Americas coupled with high viremia in infected travelers returning from endemic areas increases the risk that this virus could establish itself in new endemic regions.

It has already been reported that insect-borne diseases, like other infective pathologies, are typically subjected to explosion outbreaks followed by inexplicable rapid disappearance of the mass phenomenon. This is particularly important for CHIKV, characterized by an alternance of emergences and re-emergences in different parts of the world. It is important to follow the itinerary and time sequence of CHIKV outbreaks as they offer exemplar behavior of an insect-borne disease. Before 1999–2000, human infections in Africa had been at relatively low levels for a number of years, but in 2000 there was a large outbreak in the Democratic Republic of the Congo. In February 2005, a major epidemic occurred in islands of the Indian Ocean, reaching a peak in 2006. In 2006 and 2007, India was mainly affected, but later several countries in South-East Asia were also impacted. In 2007, there was a recrudescence in Africa, in Gabon. In 2005–2007, India, Indonesia, the Maldives, Myanmar, and Thailand reported more than 1.9 million cases. In 2007, transmission was reported for the first time in Europe, in a localized outbreak in northeast Italy, followed by several reports in France. However, vectors were able to land in America and spread. In December 2013, France reported two laboratory-confirmed autochthonous cases in the French part of the Caribbean island of St. Martin. Since then, local transmission has been confirmed in more than 43 countries and territories in the WHO region of the Americas. This is the first documented outbreak of CHIKV with autochthonous transmission in the Americas. As of April 2015, more than 1,379,788 suspected cases of CHIKV had been recorded in the Caribbean islands, Latin American countries, and the United States of America. A total of 191 deaths were also attributed to this disease during the same period. Canada, Mexico, and the USA have also recorded imported cases. On October 21, 2014, France confirmed four cases of local CHIKV infection in Montpellier, France. Also in late 2014, outbreaks were reported in the Pacific islands. Currently, a CHIKV outbreak is ongoing in the Cook Islands and Marshall Islands, while the numbers of cases in American Samoa, French Polynesia, Kiribati, and Samoa have reduced. The WHO responded to small outbreaks of chikungunya in late 2015 in the city of Dakar, Senegal, and the state of Punjab, India. In the Americas in 2015, 693,489 suspected cases and 37,480 confirmed cases of CHIKV were reported to the Pan American Health Organization (PAHO) regional office, of which Colombia bore the biggest burden, with 356,079 suspected cases. This was less than in 2014, when more than 1 million suspected cases were reported in the same region.

In 2016, there were totals of 349,936 suspected and 146,914 laboratory confirmed cases reported to the PAHO regional office, half the burden compared to the previous year. The countries, reporting most numbers of suspected cases, were 265,000 (Brazil), 18,000 (Bolivia), and 19,000 (Colombia). The year 2016 was the first one that autochthonous transmission of chikungunya was reported in Argentina, following an outbreak of more than 1000 suspected cases. In Africa, Kenya reported an outbreak of

CHIKV resulting in more than 1700 suspected cases. In 2017, Pakistan continued to respond to an outbreak which had started in 2016.

Zika virus

The reported cases of Zika disease are relatively few in comparison with other insect-borne diseases. To understand the phenomenon associated with the Zika disease, we must introduce the word "pandemic," more commonly associated with influenza. A pandemic is the worldwide spread of a new disease. This is the main difference to an epidemic, which is a term broadly used to describe any health problem that has grown out of control. The widespread occurrence of a disease is considered when defined in a community at a particular time. In other words, the term "epidemic" should be preferred in the case of a disease actively spreading, whereas pandemic relates a geographic spread affecting a whole country or, more appropriately, the entire world. A pandemic occurs when a new virus or other infecting agent emerges and spreads around the world, thanks to the absence of efficient immunity in most people. Fear and panic about pandemics are generated with the feeling of a rapid spread, facilitated by tourism and travel, and the possible general infection of everyone. Pandemic derives from the Greek *pan*, meaning "all," and *demos* meaning "people." However, if for influenza vaccines offer an efficient form of defense, for insect-borne diseases the situation is less favorable. Alarms and alerts about outbreaks are therefore the result of a mass emotive reaction by the population, due to the possibility of transmit immediately and everywhere information. Globalization is surely part of pandemic alerts. As clearly evidenced in the COVID-19 (SARS-CO2) outbreak, the emergence of a new strain of virus, as well as its consequences, is not predictable. Every measure is important and it is not possible to decide a priori if the counteractions are adequate or consequences of an exaggerated impulsive reaction.

The Zika virus is the cause of the Zika disease, and is a *Flavivirus*. The vector of Zika virus is an *Aedes* mosquito, which can bite monkeys and humans, with *Ae. africanus* and *Ae. aegypti* as the principal probable vectors (Benelli, 2016). Symptoms generally include fever, mild headache, aches, joint pains, maculopapular eruptions, and rashes in different parts of the body. Most of those affected not experience any symptoms at all, but in certain cases the consequences can be more significant. The Zika fever is common in West and Central Africa, but it also occurs in many Asian countries, and since 2014, its presence in American regions has been reported. The very few cases identified in the United States were considered travel-associated and in 2018 and 2019, there were no reports of Zika virus transmission by mosquitoes in the continental United States. It is possible that the Zika fever was overestimated, as in the case reported here, but this most recent intruder in insect-borne diseases rapidly acquired the status of a global threat, attaining higher virulence and causing complex clinical manifestations, including microcephaly in newborns, infants, and babies, and Guillain-Barré Syndrome. Owing to its rapid spread and the dissemination of information about possible consequences, a general alert raised public concern and generated panic and fear.

However, most of the world did not know about the existence of the Zika virus and its danger and potential impact until the 2016 Summer Olympic Games in Rio de Janeiro. The situation generated a debate about the danger for approximately 16,000 athletes and 600,000 visitors coming from abroad to the Games. Data confirmed the potential risk in the form of the state of Rio de Janeiro recording 26,000 suspected Zika cases, the highest of any state in Brazil, with an incident rate of 157 per 100,000, the fourth highest in the country. Everything started in December 2014 with the first reports of an unknown exanthematic disease outbreak in Brazil. Later, the disease was identified as the Zika infection. In May 2015, the spread of the disease to other parts of the country, first in the states of Pernambuco (PE), Rio Grande do Norte (RN), and Bahia (BA) in the northeast region, then in other states of the central-west and southeast regions. Again, geography gives us some help in interpretation. The state of Pernambuco is in the extreme eastern part of Brazil, projecting out to the Atlantic Ocean and practically in front of Africa. The state is part of the *sertão*, a very beautiful equatorial place with splendid *playas* full of *coqueiros* in the coast, but the internal part has needed to face the progressive desertification in the last decades (*sertão* in Portuguese means great desert). The rains could be enough, but they are concentrated in a short period. In recent years, this situation generated a massive migration toward the coast with the major towns Recife and Macejò, causing usual conditions for the development of infectious diseases and their progressive expansion. An outbreak is usually a combination of factors, like a massive susceptible population, climatic conditions conducive for diffusion of the mosquito vector, socioeconomic impact, and alternative nonvector transmission vehicles. However, this rapid spread was probably due to the special characters of the spread of this disease, in comparison with other ones, including sexual transmission. In fact, dengue virus took several decades to diffuse, bound by a climatic barrier in the south and low population density areas in the north, while the Zika virus epidemic rapidly spread across Brazil within months, and beyond the area of permanent dengue transmission. Thus, Zika spread rapidly across Brazil and to more than 50 other countries and territories on the American continent. Its speed was one of the key factors for the epidemic alert, leading Brazil to declare a national public health emergency in November 2015, confirmed by the World Health Organization three months later, and culminating in an explosive situation involving a decision about the Olympic Games. The debate was furious, being without any reliable results, as is common when science is mixed with economic and political interests. Again the WHO was asked and responded to the claims, evidencing the general low impact of the Zika virus, resulting in mild symptoms if at all in those affected, opening the door to the Games with its authority. In effect, the epidemic was progressively subsiding, thanks to Brazilian efforts to mitigate the risk to athletes and visitors by a series of indications to take precautions in protecting themselves from mosquito bites and practicing safer sex. This is another indication that the agent cannot be considered totally guilty of the epidemic, but the social situation, with absence of hygiene and adequate control, are necessary concurrent causes. The parasite does its homeostatic work, and humans amplify this. Therefore, something changed.

2016–2018: According to WHO data: "incidence of Zika virus infection in the Americas peaked in 2016 and declined substantially throughout 2017 and 2018." In fact, due to its pandemic potentiality, the Zika disease is the most carefully detected one by international and local heath institutions. This tendency can be confirmed in the following timetable of the Zika outbreak.

2018–2019: The Pan American Health Organization (PAHO) in Brazil reported 19,020 cases of Zika disease, of which 1379 were laboratory-confirmed. In 2019, as of week 9, the Brazilian Ministry of Health reported 2062 probable cases, compared to 1908 cases reported over the same period in 2018.

2018: PAHO, in addition to Brazil, reported data on other countries in South America, with the majority of cases in Peru (984), in Bolivia (1736 with 486 laboratory-confirmed), and Colombia (857, laboratory-confirmed). For the whole of 2018, Mexico reported 860 confirmed cases of Zika disease, an evident decrease in cases compared to 2017, at 3260. In 2018, Guatemala was one of the countries much affected with 2300 ZIKV disease cases being notified during 2018, compared to 703 cases reported in 2017. Of these cases, 106 were confirmed in 2018 and 164 in 2017.

2018–2019: In 2018, El Salvador reported 481 cases of ZIKV disease, against 450 cases in 2017 and 128 Zika disease cases, in 2019, as of week 11, compared to 66 cases for the same period in 2018. In March 2019, Peru and Colombia remained among the countries with the highest numbers of cases, with 275 and 110 cases, respectively. In Central America, Mexico reported 13 confirmed cases, compared with 39 for the same period in 2018.

2019: No vector-borne locally disease cases had been reported by EU/EEA countries in Europe as of week 12 in 2019, whereas in the period 2015–2019, 22 EU/EEA member states had reported 2398 travel-associated infections through the European Surveillance System.

The diffusion of Zika due to travel and tourism is carefully detected in the USA, but a significant decrease in travel-related cases returning to the continental United States has been observed for the same period, from 4897 cases in 2016 to 72 cases in 2018. In December 2017, it was possible to find the most recent mosquito-infected autochthonous case in the continental United States, as reported in Hidalgo County, Texas. Since then, and as of March 6, 2019, no autochthonous mosquito-borne transmission had been reported in the continental United States. Data from the other parts of the world confirm these trends.

Finally, Zika, despite general concern, is not presenting a current menace, but it can be considered an example of positive control of an insect-borne disease when appropriate attention is exercised, although the social effects should be considered and amplification by information limited if possible.

Unfortunately, considering previous experiences in other insect-borne diseases, this trend cannot be considered reliable. However, we can trust reliable diagnostics for detecting the Zika infection and several research advancements in drug/therapeutic targets and identification of vaccine candidates. Despite these research achievements, currently there is no effective drug or any vaccine available against the Zika fever.

Yellow fever

There are several similitudes with the dengue disease. Again, the agent belongs to the *Flavivirus* genus and it is present in tropical areas of Africa and South America. Remember that the word *flavus* means "yellow" in Latin and the yellow fever in turn is named for its propensity to cause yellow jaundice in infected people. Again, *Ae. aegypti* is the most important vector, at least in America. Monkeys are the main reservoir of infection; by transmission from monkey to monkey, the virus is transmitted and can infect humans, with a vector change. This disease is the cause of 200,000 clinical cases and 30,000 deaths every year. A total 90% of cases are reported from Africa, mainly the Sub-Saharan regions, and these predominantly affect young people. However, also in this case these data must be considered indicative, since most infections and deaths occur in rural areas, where surveillance and reporting can be inadequate. In the Americas, the disease is distributed from Philadelphia in the USA to Mendoza in Argentina. The disease can be fatal, acute, or mild, and even unapparent. There is no antiviral treatment, but it is possible to prevent yellow fever infection by vaccination.

West Nile fever

Again, this disease is caused by a virus of the genus *Flavivirus* and the vector is an *Aedes* mosquito, aided in this case by *Culex* (Aziz et al., 2015). The disease has been reported in regions of all populated continents, including the whole of America. The infection effects can be invisible, or cause mild fever, meningitis, encephalitis, acute symptoms similar to poliomyelitis syndrome, and ultimately death. The most interesting aspect of this disease is the range of hosts involved. West Nile fever can infect birds, horses, and humans, with different signs and symptoms, including none at all, until the point of death. The first cycle of the virus concerns several bird species and mosquitoes, supporting replication of the virus. The other targets of the virus are mammals, mainly horses and humans. Although in horses the cyclical disease is characterized by mild to severe symptoms of ataxia, involving weakness, muscle twitching, and nerve deficiency, including cranial damages, in humans most infections are asymptomatic. However, in the 1990s, the disease became an important human and veterinary challenge. The impact of the disease increased noticeably in human infections. People over the age of 50 are more at risk and can develop serious forms of this disease. The problem is that the incubation period of this disease can cover several weeks and approximately 80% of infected people are totally asymptomatic or symptoms can be confused with others in aged patients. In fact, in many developed countries, vector-borne tropical diseases are so rare that the experience of medical doctors to diagnose these diseases became inadequate in the absence of direct experiences. A vaccine is available, but only for horses.

A similar case concerns St. Louis encephalitis, a viral disease transmitted by *Cules* sp.; birds are usually the hosts, but it can also affect humans and other hosts. This disease is currently present in Canada, Mexico, and Central and South America. The clinical symptoms of this encephalitis are often not manifested, except during epidemics, wherein children and elderly are the most susceptible, with a mortality is 5%–20%. No vaccines or other effective treatments are available, including antibiotics, because they are not effective on the virus. Similar diseases are eastern equine encephalitis virus and western equine encephalitis, wherein again birds, mosquitoes, horses, and humans are involved. Again, mortality is between 5% and 15%, and infants, children, and elderly people are most affected. Around 50% of survivals result in severe permanent brain damage. There is no effective vaccine for humans so far.

Lyme disease

Lyme disease, also known as Lyme borreliosis, is probably less famous compared to West Nile fever, but it has been described by the *New York Times* as "the infectious disease able to diffuse most rapidly worldwide after AIDS." There are several reports of its increasing diffusion in Asia, Europe, and South America. Lyme disease is the most common disease spread by ticks in the Northern Hemisphere, with an estimated 300,000 infected people a year in the USA alone, and 65,000 people a year in Europe.

The name of this disease relates to he town of Old Lyme, Connecticut, USA, where in 1975 the first epidemic was reported. According to the reports, in Lyme a mysterious increase of arthritis cases occurred, especially in infants, causing at first apparently innocuous cutaneous erythema on the thorax and other parts of the body, which are considered symptoms of juvenile rheumatoid arthritis. However, in contrast to this disease, the symptoms persisted with increase of the erythema, associated with fever, headache, joint pains, and tiredness. The acute form of the disease includes loss of the ability to move one or both sides of the face, joint pains, neck stiffness, and heart palpitations, among others; shooting pains or tingling in the arms and legs, memory problems, and tiredness can also occasionally develop. Months to years later, repeated episodes may occur.

The infection is caused by a bacterium of the genus Borrelia, spread by ticks, but it is transmitted to humans by the bites of infected ticks of the genus Ixodes. Approximately 70%–80% of infected people develop a rash, which is typically neither itchy nor painful.

As for other arthropod-vectored diseases, infections are most common in the spring and early summer. Beside prevention and measures of control, research is ongoing to develop new vaccines. An effective vaccine for Lyme disease was marketed in the United States between 1998 and 2002, but it was withdrawn from the market due to poor sales.

Other vector-borne diseases

It is necessary to mention Japanese encephalitis (1E)-epidemics, whose incidence has been reported to be particularly high among pediatric groups with high mortality. Nearly 1.4 billion people in 73 countries worldwide are threatened by lymphatic filariasis, a noxious parasitic infection that leads to a disease commonly known as elephantiasis. Filariasis is vectored by mosquitoes, with special reference to the genus *Culex*. The main control is currently against mosquito larvae and consists in the treatment with organophosphates and insect growth regulators, but with negative effects on human health and the environment. Green-synthetized nanoparticles have been recently proposed as highly effective larvicidals against mosquito vectors, whose details can be found in Chapter 7, dedicated to new solutions.

Insect-borne diseases affecting animals

Metamorphoses in the food environment

The food planetary situation is full of contradictions. On one side, the alimentary systems—consisting of production, transformation, distribution, and consumption—have the potential to nourish the global population, in terms of quantity and quality, as well obtain the respect of the environmental sustainability. However, despite this positive potentiality, food production currently still appears in the opposite direction. Although food world production in calories is proceeding in accordance with the growing population, at least 820 million persons do not have sufficient food, and even more are consuming food of low quality, insufficient in micronutrients and essential amino acids. The environmental impact is modeling the planet in the Anthropocene Age, with something like 40% of the soil designated for production of food and consumption of 70% of available fresh water. The conversion of natural ecosystems into pastures and arable lands is affecting the species equilibria in any habitat. About 33% of hydric stocks is overexploited and more than 60% is used up in production. These and other congruent data were published in the EAT-Lancet Report, named "Food in the Anthropocene" and produced by a selected pool of experts. The report also contains a series of precise indications, expanding the concept of planetary boundaries to identify a useful compromise between production and sustainability in the coming years, to meet the challenge of an estimated 10 billion inhabitants by 2050. In other words, we know what we need to do as a species, but actually making this happen is another matter.

In this part of the chapter, we will focus on farm animals and their products, in consideration of the economic importance and the strategic relevance of agricultural and livestock production (Murugan et al., 2016). This argument must be based on two considerations, the quantity and the quality of food, which are usually not coincident. The first aspect is strictly related to insect-borne disease, due to the massive use of chemicals against pests to protect production in pre- and post-harvesting steps. The second aspect evidences several changes in the production and consumption of food, with massive orientation of consumers on certain types of nourishments.

In Italy (excluding several forms of allergy to one or more food components), something like 6% of the population are vegetarian or vegan. Even vegans can be taxonomically classified into relative and absolute vegans, on the basis of their ethical considerations, refusing not only foods like milk, eggs, and honey, but in the case of sexual vegans, which avoid physical contact with omnivorous, because they are considered impure being in contact with meat. The alimentary tribalism is considered a form of recent tendency, but it is an old story. The refusal to eat meat, in particular bloody meat, can boast ancient roots. In the hypothetical Golden Age, mankind was happy and naïve, eating everything nature spontaneously offered in great quantities; only in the Silver Age did meat enter mankind's menu. Later, discrimination among foods was seriously carried out and considered necessary. Practically every religion contains a detailed list of foods to be avoided. Several of these prohibitions have historical dietetic reasons, but warnings of different kinds survived for centuries and are still present in all current religions.

Nowadays, an alimentary tendency is not a selection of the right proteins, but adhesion to a living style, indicating not a physiological aspect but a philosophical choice. Food considered as identity of a selected group, in order to be safe and purified by obesity and fats, like a necessary deprivation against abundance and obtain a soul purification. Avoiding certain foods, it is possible to maintain an evidence of separation and identity against a world dominated by the globalism. The famous sentence of Feuerbach can reloaded. We are not what we eat, but what we do *not* eat. This phenomenon is important, but still marginal. There is still 96% of the population waiting for their meat input. The need for such high production leads to massive breeding and shifts the attention away from the quality of food. If we aspire to eat better food, we must consider the production steps. Most of the meat that we eat derives from intensive breeding, where the animals are allowed to live only for the duration of their growing period and in abnormal conditions. Only the quantitative result is considered. In chicken breeding, the conversion between feed and growing should reach more than 50%, which means that for every 100 g of food, at least 50 g remains inside the animal. After 4 weeks, the chickens are ready to be converted into meat and consumed in different forms. The main problem is that chickens are confined in a small overcrowded space, where the light is always on to avoid continuous mortal attacks inside the population. In such challenging conditions, these organic machines of conversion of vegetable matter into meat need continuous human assistance because diseases and epidermics are unavoidable.

The health of animals in industrial breeding, like chickens, fish, cattle, pigs, and others, needs to be continuously medically supported, in particular in the earliest stages of growth. The result is that the meat that we eat is full of estrogens, hormones, and antibiotics, usually significantly above the suggested limits. The problem is that the antibiotics pass through the alimentary chain, are transferred in the marketed products, and eventually reach us and are accumulated by us. This connection between food and feed is already evident in many sectors. Fish consumption is nowadays deeply dependent on aquaculture. Again, production is supported by heavy utilization of antibiotics, but this will not be possible in the future.

After a long period of total confidence in antibiotics' power and consequent enormous benefits for mankind, things are changing. The continuous appearance of new bacterial resistant strains is anticipating the postantibiotic era, when antibacterial drugs that currently work will be inefficient. Many fish producers, supported by legislation, are already partially replacing antibiotics with selected natural products introduced in food, in order to increase natural immune defense, and important results are being obtained.

Other breeding sectors are following their example. This could be the compulsory future of breeding and meat production, not considering the already present and increasing market of ecologically produced products. However, it will be a long road before things really change.

The situation concerning animal farming and derived products is every day worse. In industrial farming, animals are considered not as living organisms, respecting their lifestyle and environmental needs, but simply a method of conversion of food into other foods, generally food for animals into food for humans. Not considering the extreme conditions, here again there is a continuous input of antibiotics, but we must consider the epigenetic factor. When we change radically the environmental conditions of animals, we are producing animals very different from the starting ones, because of the accumulation in DNA of small but significant changes. The changes are translated in the composition of the derived products. Therefore, the present nutritional input is very different from previous input, and this must be considered as the cause of several consequences in the alimentation chain and at the end in our physiological equilibrium, and the source of many modern diseases (Kant et al., 2015; Donin et al., 2018).

Food for a global population of 7 billion humans

Livestock production is growing rapidly as a result of the increasing demand for animal products. Global meat production and consumption was predicted to rise from 233 million tonnes (2000) to 300 million tonnes (2020), and milk from 568 million tonnes to 700 million tonnes over the same period. Egg production was also predicted to increase by 30%.

This forecast shows a massive increase in animal protein demand, required to satisfy the increased human population. The considerable and growing demand for animal protein is focusing attention on the sources of feed protein and their suitability, quality, and safety for future supply.

Significant increases in global demand for livestock products will clearly require increasing amounts of feed protein supplies, and sources and alternatives will need to be reviewed continually. There would seem to be strong justification for research and development investment into a number of very promising new sources. What is certain is that there will need to be considerable increases in feed manufacture, requiring a thriving, successful, and modern feed industry.

Safety issues will remain paramount in the minds of consumers following recent food crises, and continuing investment is needed into quality assurance programs to gain market access for animal products and retain consumer confidence.

How we feed and treat animals intended for human consumption raises important public health concerns. These concerns arise not only from what is fed to animals but also from gaps in regulations and systems intended to ensure the safety of feed and the food supply. A variety of substances, including many waste materials from the agriculture, food, and rendering industries, are "recycled" into feed for food-producing animals. Some of these ingredients used in feed, particularly those from animal and mixed sources, may result in unwanted feed contaminants or have other unintended consequences. Animal feed plays an important role in the cycling of dioxin, arsenic, pathogens, antibiotic-resistant bacteria, prions, and other substances of public health concern. These problems have drawn attention to feeding practices within the livestock industry and have prompted health professionals and the feed industry to scrutinize closely feed quality and safety problems that can arise in foods of animal origin, as a result of animal feeding systems. Given the direct links between feed safety and safety of foods of animal origin, it is essential that feed production and manufacture be considered as integral parts of the food production chain. Feed production must therefore be subject, in the same way as food production, to quality assurance including feed safety systems.

However, assuring feed/food quality and safety is only part of the problem, because ensuring animal health and welfare and reduction of environmental impact are also fundamental. It is estimated that there are 5×10^6 EU farmers, 130 billion animals for food production, and 450×10^6 tons of feed yearly. Since the BSE-related 2000 ban of animal proteins in the EU, soy meal represents the world's best protein. However, self-sufficiency of EU country members is low (2%), resulting in a 10% increase of soy consumption and the addition of pure amino acids. Sources of protein for animal feeds are many and varied, with considerable opportunities for further diversification and substitutions. More research is required on alternative sources before many of the opportunities can be exploited in practice.

Quality protein can be provided sometimes from various crop residues and by-products of food and drink manufacture, such as brewers' grain and maize gluten meal. These by-products are many and varied, and differ considerably in the value and significance for animal feed protein supply. Today's technological capacity for reusing and further processing of these waste materials is limited due to poor knowledge about potentially useful primary and secondary plant products that are still contained in the residues. Food is lost or wasted throughout the supply chain, from initial agricultural production down to final household consumption, often only partially considering the quantity of daily produced organic waste for families. In medium- and high-income countries, food is wasted significantly at the consumption stage, as it is often discarded even if it is still suitable for human consumption. Significant losses also occur early in the food supply chains in industrialized regions. In low-income countries, food is lost mostly during the early and middle stages of the food supply chain due to deterioration, whereas much less food is wasted at a consumer level.

However, some of these by-products provide a valuable local source of protein, which can be inexpensive, accessible, and continuously available from the local food industry. Their use can also be regarded as a significant recycling opportunity, and more of a closed system for waste disposal. Food safety considerations may still dominate this protein supply route, with restrictions on certain by-product materials or their treatment before use in animal feed. Production of meat and other livestock-derived products can be highly affected by insect-borne diseases. Medical treatments are crucial for meal production and animal welfare, and these are strictly related to antibiotics and insect-borne diseases, but preventive counteractions are once again necessary and decisive. Regarding the insect-borne diseases affecting animals, a single report is evidenced here and this case utilized as a model of current and possible devastating diseases interesting livestock. The sequence of reported events, as well as interpretations and evaluations, may be a starting point to predict and successfully face the emergence of devastating vector-borne pathogens.

Bluetongue disease

Chronology of bluetongue virus (BTV) spread in Europe

1969: First isolation in Greece of strain BTV-9 and BTV-1.

1998: Isolation in Tunisia of the strain BTV-2, endemic of Sub-Saharan Africa, and belonging to strains from South Africa, Nigeria, Sudan, and the USA.

2000–2001: Isolation in Greece and Turkey of the European strain BTV-1, similar to viruses that have been isolated in India. First outbreak in Bulgaria.

2003: A new strain BTV-4 type, different from that of Greece and Turkey, is isolated in Corsica, Sardinia, and the Balearics.

2004: Detection of strain BTV-4 in Sicily and France.

2005–2014: Distinct strains are still entering in Europe, affecting at least 12 countries and more than 800 km further north than before.

2014: In Sardinia, BTV caused the deaths of 13,000 sheep and damage costing 42 million euro. A total of 5772 infection sites were detected.

Bluetongue (BT) is a vector-borne disease of domestic and wild ruminants caused by viruses of the genus *Orbivirus* transmitted by *Culicoides* midges (Bowne, 1971). BT is a complex multihost, multivector, and pathogen disease, whose occurrence is fluctuating, re-emerging after long periods of absence, when most transmission occurs silently in disease-resistant animals, and short devastating phenomena affecting in preference certain breeds of sheep, which are economically very important for production of high-quality wool (Saegerman et al., 2008). Cattle are the most common reservoir, being able to be subclinically infected also for prolonged infection. The disease's name stems from the most evident manifestation of the disease, a typical blue coloration of the tongues of infected animals.

The disease manifests a pre-patent period of 3–8 days. The symptoms in sheep consist of transient fever (up to 106°F or 41°C), edema of the face, lips, muzzle, and ears, excessive salivation, and hyperemic oral mucosa. Later, at 7–12 days, several lesions may progress with petechial hemorrhages, erosions, and ulcers; a marked pulmonary edema is also often seen. Wool became fragile and no longer useful for the market. As a consequence of severe oral lesions, animals are reluctant to eat and suffer acute diarrhea, and muscle and coronary band pain limits movements. Finally, many affected animals become depressed and die, although others fully recover. Although the target of this disease is limited to sheep, cattle, and some species of deer and camelids, the effects can be epidemic and devastating, causing alerts for immense economic damages. While BTV first appeared in 1998, a transmission and maintenance network has recently emerged in Europe, causing the deaths of more than 1 million sheep, which are considered victims of viremias caused by BTV, with annual losses of US$125 million in the USA alone in trade of animals and animal products. In recent years, the livestock industry in the USA suffered he deaths of approximately 179,000 sheep within 4 months caused by epizootics of BTVs. Decreased trade associated with BT outbreaks has become an even greater threat to the livestock industry than that of the disease itself, becoming a major obstacle to exports of U.S. ruminants and ruminant products.

The virus

BTV includes viruses of the genus *Orbivirus* pertaining to the family Reoviridae, which is a large group of double-stranded RNA viruses with 10–12 genome segments (Howell and Verwoerd, 1971; Verwoerd et al., 1972). The group is complex and varied, as consequence of a strong capacity of adaptation to the environmental changes. BTV cross-reacts with many antigenically related viruses including Palyam virus and the viruses that cause epizootic hemorrhagic disease and African horse sickness. BTV replicates in both arthropod and mammalian host cells. The virulence of BTV varies markedly; even strains with matching serotypes have variable virulence. A total of 26 serotypes of BTV have been identified worldwide with testing through viral antigens. The tests indicated great differences in the distribution since only five have been recognized within the USA and at least nine in Europe.

BTV presents interesting peculiarities. Its structure was found to be the same as that found in reovirus, consisting of a similar segmented, double-stranded RNA, with even polypeptides present in the capsid, four of which are major and three are minor components. However, Verwoerd et al. (1972), suggesting a direct coding relationship between the genome of the two taxa, also evidenced differences in genome segments, which may be utilized to code for certain capsid proteins. In fact, two of the BTV polypeptides are present as an outer diffuse protein layer surrounding the capsid. The authors speculated that "this outer layer probably has some of the functions of the reovirus outer capsid, one being the 'masking' of a viral transcriptase which could be demonstrated after its removal."

The vector

The virus is transmitted by certain biting species of *Culicoides* midges, pertaining to Diptera, Ceratopogonidae. *Culicoides* species total more than 1400, which are divided into many subgenera, although the classification and taxonomy of the genus are controversial and confused. Several species are known to be vectors of various diseases and parasites, which can affect animals, but less than 1% are considered responsible for transmission of BTV (Table 5.6).

C. imicola is considered the most common species vector in Southern Europe and has been recorded in Africa, Asia, and Europe so far. However,

Table 5.6 Scientific classification of the main vector of BTV in Southern Europe.

Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Diptera
Family	Ceratopogonidae
Subfamily	Ceratopogoninae
Genus	*Culicoides* Latreille, 1809
Species	*Culicoides imicola* Kieffer (former name *C. pallidipennis* Carter)

there are reports of other suspected BTV vectors, such as *C. pulicaris* and species in the *C.* (*avaritia*) *obsoletus* complex. There are also differences concerning distribution and habitat, in particular the environmental factors. In Sicily, the species *C. imicola* resulted not present in undulated areas of high altitude. This was related to the very hot summers of the island, in particular in inner areas, because in highly angled undulating topographic areas, rapid desiccation leads to drying of soil, which prevents proper larvae development. Further examinations have indicated that a suitable climate and nutritious soil must be considered the biggest determining factors in distribution, rather than altitude. A likely reason is that *C. imicola* pupa are especially prone to drowning, so their eggs are often laid in surfaces free of running water. Therefore, as already reported the larvae, as with other early stages of the insect, need moist soil, so there tends to be a trade-off between dry and wet areas.

BTV was first described in South Africa, where it has probably been endemic in wild ruminants since antiquity. Since its discovery, BTV has had a major impact on sheep breeders in the country and has therefore been a key focus of research at the Onderstepoort Veterinary Research Institute in Pretoria, South Africa. Several key discoveries were made at this Institute, including the demonstration that the etiological agent of BT was a dsRNA virus that is transmitted by Culicoides midges and that multiple BTV serotypes circulate in nature. Multiple serotypes circulate each vector season with the occurrence of different serotypes depending largely on herd immunity. Indigenous sheep breeds, cattle, and wild ruminants are frequently infected, but rarely demonstrate clinical signs, whereas improved European sheep breeds are most susceptible.

After a long period, characterized by sporadic incursions in some areas of Europe, resulting in the vector being confined to tropical and subtropical areas, the situation changed in 1998. The emergences in Europe of several new virus strains were reported and carefully monitored. Sero-surveys showed that virus strains were closely related to those that were previously circulating in the same regions, but more virulent. So far, we were not able to determinate the factors responsible of such genetic changes in this RNA arbovirus; however, it is clear that some small changed occurred somewhere in the Middle West selectively, probably independently and/or simultaneously. Otherwise, the changes could be manifested in limited strains and transmitted to others by the mechanisms already reported, causing a spectacular migration of BTV. The migration was carefully monitored by sequence analyses and it is considered a consequence of the climatic changes of the last decades in Europe. It is noteworthy as in this case the monitoring and the relative map of pathways were fully determined and clearly reported, whereas in other cases of insect-borne diseases affecting plants, like that further reported, there is total confusion. In any case, monitoring is fundamental to understand the developing of epidemic episodes and respond, possibly by prevention. This is possible nowadays thanks to powerful and appropriate techniques and devices, but their use is often limited or even absent. Research institutes and universities should be heavy involved in such monitoring. The current regime of large freedom in investigation items should be changed in favor of studies dedicated to the general interests, with the necessary capacity of prevision of outbreak and of the sanitary system to react. Therefore, the basic research should be combined with the current needs to obtain information and data about the going on of the phenomenons interesting the public concern.

From some point on the geographic map in the Orient, the migration of strains of BTV started to conquer new territories following two independent pathways (Pioz et al., 2012; Purse et al., 2005). Analyses followed and evidenced the routes of arbovirus in the conquest of southern regions of Europe. An eastern group, which resulted in viruses similar to those isolated in India, moved laterally toward the Occident and was able to cross North Africa through the African Mediterranean coasts. In this way, it arrived on the coast of Tunisia and finally crossed the sea and landed in Europe, proceeding by three directions of expansion: the Balearics (Spain), Sardinia/Corsica, and Sicily/Calabria. In particular, the BTV-4 strain was involved, but before leaving Africa it joined with another serotype, BTV-2, coming from Central Africa. It has been reported that BT is endemic throughout most of South Africa, since 22 of the 26 known serotypes have been detected in this region. Later, parts of southern regions of Italy were also involved.

The adult *Culicoides* insects are not valid flyers, but they can be easily dispersed by the wind in passive journeys for hundreds of kilometers, in just a few nights. Otherwise, they can be transported by the routes of ruminants, as results of animal trade. These routes are like consolidated "corridors" from South Asia to Europe, formed by the connected ruminant population, usually passing through Pakistan, Afghanistan, Iran, Turkey, and beyond.

Therefore, distance is not a problem, and so far the diffusion was mainly not allowed by adverse climatic conditions. A second route, involving the spread of the "western" BTV-1, −2, −4, and − 9, passed through Anatolian Turkey, Greece, the South Balkans, then Albania, crossing Mediterranean Sea and reaching the Apulian peninsula (Melior and Wittiman, 2002). It is interesting to note how these routes are exactly the same used over the years by human migrants to reach Europe, landing in Italy after a desperate and dangerous navigation with any kind of craft or through a long terrestrial trip without any help or support.

Changes in climate conditions in Mediterranean countries could made possible stable introductions, driving the spread of vectors and pathologic agents (Mann et al., 2009). The environmental effects must be included in programs of control completed by data to understand vector behavior and distribution, seasonal abundance, and infection rates, with the aim to predict disease incidence and spread across coastal and inland areas, in order finally to define their role in virus overwintering, as reported in the following case.

In this regard, it is important to connect Culicoides vector's life cycle and the environmental factors. After the three initial stages (eggs, larvae, and pupae) highly affected by the availability of moisture, since semiaquatic habitats are necessary locations for larvae, pupae, and early adults, environment changes and adults are able to look for better places. Later, the adult females begin egg maturation to obtain the oviposition and restart the cycle. During the maturation of the eggs, named the gonadotropic cycle, the female looks for blood-feeding and, in the case of infected blood, starts the cycle involving the host's participation. Therefore, the first cycle is concentrated on homeostatic development and survival of the vector, whereas the second one is a consequence of the need for reproduction, consisting of the BTV transmission cycle. Let us start the cycle with a susceptible ruminant, which develops a viremia and is infective to the vector during the so-called latency period. After 2–4 days, an uninfected adult bites the infective host in search of blood. In this stage, called the extrinsic incubation period, the virus can enter the midgut of the Culicoides, with dissemination through the hemocoel. This is a large body cavity that is formed from an expanded "blood" system. The result is the consequent infection of the salivary glands of the host. After 4–20 days of incubation, the infected insect will bite a susceptible target, causing infection and restarting the cycle. The real effects of the disease occur during the extrinsic incubation period. Attention must be focused on the efficiency of the superorganism, as a complex system, and the possible consequences in the realization of some crucial passages. The efficiency of the virus's dissemination is dependent on temperature, influencing vector competence and rate of virogenesis, as well as other stages of the vector's life cycle. There are many sensitive steps, but the superorganism generally works, albeit we experience only its positive achievements. Every times, survival of the precedent steps is in peril.

Larvae can have enormous difficulty surviving winter's cold months (or the contrary if temperatures are gradable), and adult activity rates can also be affected. The same consideration can be extended for the availability of moisture and the persistence of the BTV between seasonal vector transmission in the overwintering period.

Bluetongue epidemic in Sardinia

Since 2000, the Mediterranean island of Sardinia island has been affected several times by BT epidemics (Calistri and Caporale, 2003). Sardinia is the second largest island in Italy and in the Mediterranean Sea, and it is positioned exactly in the middle of the sea. However, despite its position, in contrast to Sicily, Sardinia has rarely been subjected to invasion since Roman times. The reason for this is mainly related to the pride of its people, always defiant in the face of any kind of submission. This characteristic is often related to the harshness of Sardinia's central mountains. Sardinia has its own language (not a dialect) and is famous for its splendid seaside, but also for the production of a special cheese, named pecorino, since it is made by the sheep milk (*pecora* is sheep in Italian). Therefore, sheep are from ancient times one of the most important economic resources in the economy of the island. In 2018, the number of sheep and goats in Sardinia was estimated at 3.7 million: 93% sheep, meaning more sheep that inhabitants, and the remaining 7% goats. In the same year, the presence of serotype BTV3 was reported again after a period of absence. The animals affected were very few, but sufficient to create an alert. In that year, the sheep deaths accounted for 29,302 and 2500 farms were interested, accounting for 824,620 involved animals. Therefore, BT is a nightmare for Sardinian shepherds despite the compensation received for the damages suffered.

The immunization of susceptible sheep remains the most effective and practical control measure against BT. In order to protect sheep against multiple circulating serotypes, three pentavalent attenuated vaccines have been developed. Despite the proven efficacy of these vaccines in protecting sheep against the disease, several disadvantages are associated with their use in the field. In Sardinia, live-attenuated (modified live virus, or MLV) vaccines against BTV, first developed in the USA, are available and are used to control BTV. In small ruminant livestock, MLV vaccines provide good protection and limit the damages, although they were not able to solve the problem totally against clinical disease following infection with the homologous virus serotype. These MLV vaccines must continue to be used in the immediate future for protective immunization of sheep and goats against BT. Two doses of MLV (in some instances, just one dose) can provide substantial immunity to the epizootic serotypes of either BTV. Again, monitoring is fundamental, because vaccination should be provided to the field within 2 years of the initial incursion. However, it is recognized that this is not the final solution, and further research and development are warranted to provide more efficacious and effective vaccines for control viruses transmitted by biting midges, like BTV and epizootic hemorrhagic disease virus (EHDV) infections. EHDV is another economically important virus that can cause severe clinical disease in deer and, to a lesser extent, cattle. Until the recent climate changes in Southern Mediterranean, about the control strategies in fringe regions, the utilization of EHDV was limited to Turkey, Cyprus, and Israel, which are the first countries interested in the migration routes. However, vaccination did not solve the problem completely, and other actions should be taken.

Meanwhile, other solutions have been proposed. A research project was performed to deal with BTV diffusion by the utilization of natural products, in particular the neem. Although vaccination can give results, the problem of controlling the disease remains. Once again the target to obtain preventive results is the vector, and in particular the stage of the insect when it is easier to limit proliferation. *Culicoides*, like many other mosquito vectors, prefer to populate moist microhabitats, like irrigation channels, drainage pipes, and dung heaps. In particular, they can often be found in drinking troughs or water pools, where sheep and cattle drink water during the day, especially in summer (Fig. 5.17–5.19).

Fig. 5.17 Typical drinking trough for sheep and cattle.

Fig. 5.18 Irrigation channel frequently used by cattle to drink water.

Fig. 5.19 Another source of water.

The research of a new solution started several years ago, a long period of gestation being necessary for the experiments. My group of research is integrated by entomologists of the University of Cagliari, Sardinia, with long experience of BT. A previous research, involving virus detection in insect body pools of the prevailing captured midge species and supplemented by specific body region analyses, evidenced the serotype BTV-1 and BTV-2 in Sardinia. However, although *C. imicola* represents the main recorded BTV vector, other European *Culicoides* biting midges turned out to be possibly implicated in virus transmission in Sardinia. The *Newsteadi* complex, based on *C. newsteadi* species A and species B, prevailed for 47.7% of the biting midge species, followed by *C. imicola* (27.8%) and the *Obsoletus* complex (*C. obsoletus* and *C. scoticus*) (17.6%), with a territorial distribution that confirmed and completed the French consideration. While *C. imicola* was more abundant along coastal areas, the *Newsteadi* complex was frequently collected at higher altitudes and the *Obsoletus* complex was notably associated to cattle farms. Furthermore, the analyses of thorax and head, containing salivary glands, of the vectors, evidenced higher infection rates associated with *C. scoticus*, *Newsteadi* complex, in comparison to *C. imicola*. The virus was detected in *Newsteadi* complex and *C. obsoletus* in winter and spring, whereas it was mainly found in summer and autumn in *C. imicola*. Therefore, at least in Sardinia, BTV is transmitted by multiple *Culicoides* vectors. These data are very interesting, showing the complexity of the BT disease, and any program of control on insect-borne disease should be primarily based on an accurate analysis of the phenomenon at the local level, since the climatic envelope, consisting of the range of climatic variation in which a species can persist, is the main factor to consider.

Field experiments were performed and the results published in 2017 in *Research Veterinary Science*. The focus of the research was the definition of an innovative program of vector control and started from the knowledge of the cycle vector and the necessity of moist sites, consisting of breeding muddy sites close to livestock grazing areas utilized by the livestock, essential for the larvae's survival. Aqueous formulations neem cake from *Azadirachta indica* (see Chapter 7) turned out to be effective to control young instar populations of *Culicoides* biting midges (Foxi and Delrio, 2010, 2013). The field experiments were preceded by laboratory bioessays, which showed a larval lethal concentration value (LC_{50}) of 0.37 g/L of neem cake after 7 days. Furthermore, the same solution was applied directly at a dose of 100 g/m^2 on the water's edge of a pond margin of a livestock farm in Sardinia and other similar locations. The resulting data 28 days after the treatments evidenced a significant reduction of in *Culicoides* emergences. The treatment proved to be more effective in the case of *C. imicula* compared to other vector species, such as *C. catanei*, *C. circumscriptus*, and *C. festivipennis*. Therefore, neem cake can be considered useful in programs for prevention of BT outbreaks, outlining the current lack of effective tools in the fight against *Culicoides* vectors.

Insect-borne diseases affecting plants

The centrality of the nutritional environment

In all the arguments present in this book, it is necessary to realize that also in the case of insect-borne diseases, competition for food is the central aim of all activities of any living organism, and the disease is a collateral effect. In the last decades, several evolutions of the nutritional environment have changed radically the quality of food, including production, storage, distribution, and consumption. The changes in action indicate the key role of infection of plants that must be considered fundamental for human alimentation, even survival of mankind.

Nutrigenetics and nutrigenomics have demonstrated that the quality of aliments is important in prevention and on the impact of novel epidemic diseases, like globesity and related illnesses. The global nutrition environment evidences wracking contrasts and precarious equilibria, which can be derailed by environmental changes. Again, the problem of competition for food sources is fundamental. Mankind's world is divided into two parts: 520 million malnourished persons are on one side and the other side is overproduction with 20%–30% of the produced food lost or wasted, making a contribution of 8%–10% CO_2 increase only in 2010–2016. The food environmental scenario is in swidt evolution with several factors influencing the quantity and quality of food. The problem of desertification is well-known, involving the loss of precious cultivable lands, but another type of desertification concerns our everyday food (Kelly et al., 2016). Most of the millions of people living in developed countries suffer serious limited access to affordable fresh food (Karthika et al., 2016). This population, crowded into huge urban centers, creates devoted customers at hypermarkets in the weekend and consumers of ready-prepared food. They are subjected to the so-called "food desert." This desert concerns limited access to fresh whole food, like just-produced fruits and vegetables, which are considered very important for health maintenance and illness prevention. The Social Market Foundation reported that in 2018, 10.2 million people in the UK were interested in the food desert phenomenon, with 1.2 million living in deprived areas. The counterpart is an increasing consumption of ultra-processed foods, rich in preservatives, fats, salt, and sugars, but lacking in vitamins, antioxidants, and other natural products. Insect-borne diseases are involved in the storage and conservation of ultra-processed foods, since their organic materials are the ideal medium for larvae and microorganisms, which have sufficient time to work. Packaging is usually deficient, even wasteful, although recent solutions like intelligent packaging are now available.

The living conditions in towns and cities push us to the utilization of already prepared foods, determining the empires of takeaways and fast food restaurants, where the quality of food is an afterthought. The only reference is the taste of the ingested food, but nothing is known about the food's production and the origin, as well as other similar "details." The correlation between health and this kind of food has been well-evidenced by a recent cross-sectional study, indicating the risk in takeaway meals for coronary heart disease, type 2 diabetes, and obesity in children aged 9–10 years. Takeaway meals and fast-foods are known to be energy-dense and of poor diet quality. This kind of food should carry the same warnings used for tobacco products: "Fast food can cause a slow and painful death." The association between fast food and biomarkers of chronic disease and dietary intake has been clearly reported by many studies. In particular, Kant et al. stated that:

"American adults reported a mean of 3.9 (95% confidence interval 3.7, 4.0) AFH and 1.8 (1.6, 1.9) fast-food meals/week. Over 50% of adults reported $\geqslant 3$ away from home meals and $>35\%$ reported $\geqslant 2$ fast-food meals/week. The mean BMI of more frequent away from home meals or fast-food meal reporters was higher ($P\text{trend} \leqslant 0.0004$). Serum concentrations of total, LDL and HDL-cholesterol were related inversely with frequency of away from home meals ($P < 0.05$). Frequencies of fast-food meals and serum HDL-cholesterol were also related inversely ($P = 0.0001$). Serum concentrations of all examined micronutrients (except vitamin A and lycopene) declined with increasing frequency of away from home meals ($P < 0.05$); women and $\geqslant 50$-year olds were at higher risk."

The food desert is in practice a loss of precious molecular variability in alimentation, in favor of massive production of food consisting of a mixture of fats, sugars, and proteins, virtually repeated in every meal. Most of the current nutritional system is reduced in the composition to the basic nutrients, cooked in a simple and practical way, sustained by an aggressive marketing, ready to be quickly and easily consumed, without any idea about the story and the importance of what is going to be eaten.

The packaging with a beautiful picture of the food inside is the only appealing information, usually with few relation to the ingredients. This kind of food is ready to be cooked and eaten. No change or transformation is allowed, just eating. The lack of information and knowledge, including the correct scientific sensibility, is a general problem. I remember my difficulties explaining, at a professional meeting of an important U.S. government agency, that olive oil is not only a mixture of fatty acids and triglycerides, and the importance of its complex variable composition, being the result of the bio-transformation of environmental different inputs of the territory. That is the fundamental importance of the minor components, making the Mediterranean diet one of those most highly rated for health and classified by UNESCO, in 2010, as an Intangible Cultural Heritage of Humanity.

Fundamental competition for food

Although social attention is generally focused on insect-borne diseases affecting humans and the consequent medical treatments, parasites are spread all around and most economic damage concerns crops and other cultivated plants of strategic importance for alimentation of humanity and environment sustainability. Now we have clear the consequences of a human pandemia, but let us imagine the consequences of a pandemia affecting livestock or cereals, reducing totally the food's supply. In such case, there are not hospitals or medical supports and the carestia for billions of humans is the possible scenario. No available enough food means health loss and decrease of our immunity defenses. The damage and loss caused by these diseases are increasing and affecting strategic crops. However, once again the explanation of the causes of the phenomenon are quite complicated, and solutions are not as easy as might be expected.

The explanation of these diseases that we are going to use will not be different from the cases already reported. A forced alliance between very different organisms, based on the optimization of the trophic necessities of the parasite. Mankind in this contest is backstage; the negative consequences for us are simple collateral effects for the system, but enormous for the economy.

Plants are continuously subjected to several diseases wherein different organisms can be involved. As expected, crops and other economic valuable plants are mainly under investigation and considered in control strategies.

Crop and other agricultural production is expected to increase in relation to the needs of the world's population. As already reported, the global population is roughly divided between people in advanced countries facing excess of food and others experiencing difficulties in terms of limited availability and quantity of nutrients. The gap is rapidly increasing, because the second group is increasing in number and the first one is decreasing, as consequence of the globalization and concentration of production and distribution in multinational trusts. The global population is predicted to rise to 10–11 billion in the next 20–30 years, concentrated mainly in developing countries. To maintain these billions of people, so far the solution has been intensive utilization of the soil and natural sources to generate enough food, but unsustainable global situations in progress are also a consequence. Waiting for a desired equal distribution of life conditions, several aspects require immediate changes in favor of a sustainable and ecofriendly approach: new fertilizers and pest controls based on integrated activity systems.

Nowadays, agriculture also needs radical changes and brave decisions. Attention is usually focused on increasing the production, but postharvesting conditions, conservation, and waste are also very important, and these are the factors of loss of food, which are going to increase, in accordance with the production and the request. Losses due to arthropod pests currently account for around 20%–30% of the world's food production. Organic nonliving matter is a preferred target for necrophagous, parasites, and demolish organisms, in search of nutrients.

Insect damage is important in the field and in stored products, besides microbial and fungus attacks. In general, counteractions are preventive if possible, but also based on any possible way to prevent economic damage. Although it has been demonstrated that excessive and inappropriate use of pesticides must frequently be considered counter-productive, the result of the increasing needs is that the use of insecticides has increased even more than necessary.

Agricultural production resorts to the use of a large quantity of insecticides and antimicrobials to increase production and preservation of foodstuff (Govindachari et al., 1992). Effects are not limited to the treated field, involving undesirable consequences for public health and the environment. Current pesticide pollution of Adige Valley in northern Italy, due to continuous heavy treatments of apple monoculture, is a clear example. Organism genetically modified introduction was presented as an innovative and definitive solution, but its utilization induced additional problems for farmers and the market. In any case, although scientists' opinions are mostly in favor of the use of OGMs, the position of consumers is exactly opposite. Either way, the expected drastic reduction of pesticides is still unachievable.

Most insecticides are usually utilized to reduce the damage caused by insects that destroy crops or transmit diseases. Already, agriculture accounts for 59% of the resistant insect species and veterinary pests for 41%. It is important to consider that, like antibiotics in the USA and UK mainly being utilized to treat livestock being considered immunostimulants, most insecticides are used in agricultural practice to improve production and preservation of foodstuffs. In both cases, use is now widespread, excessive, and arguably inappropriate. Furthermore, we may have to face new epidemic emergencies, due to several factors, including climate changes, concerning crops and livestock. Insects are vectors of important diseases involving nonhuman targets, causing important effects on plants and animals of strategic economic relevance. Recently, some of these diseases are of increasing concern to the general population, attracting a level of attention never experienced before, and generating great alarm in terms of the consequences of their rapid diffusion. The potential economic negative effects are enormous and the damage on the local economic system could be dramatic.

So far, much attention of research and public concern has been focused on vector-borne human diseases, in order to eradicate their presence and save as many lives as possible. However, if the resistance will affect and limit the life supports, like food and water, our surviving struggle will be in balance, due to two key factors: resistance to many pesticides and clear damage to the habitat. The story already reported for resistance to antibiotics can be repeated for insecticides, adding clear damage to the habitat. Even pyrethroids are now considered dangerous, since they can impair memory and movement in nontarget animals. It is well-known that without the continuous work of pollinating animals, humanity has no future. In a new hypothetical Ark, beneficial insects should take the first available places.

All the appeals and declarations for limited and selective utilization have so far had a limited impact. Mosquitoes are the most critical group of insects in the context of public health, because they transmit numerous diseases, causing millions of deaths annually. The frequent use of systemic insecticides to manage insect pests leads to the destabilization of ecosystems and enhanced resistance to insecticides by pests, suggesting a clear need for alternatives. Most plant-based products are not as effective as their synthetic counterparts, and to apply a mosquito control in a large-scale program under epidemic conditions may be unacceptable, also considering the relative cost. Natural products of plant origin with insecticidal properties have been implemented in the past for controlling various insect pests and vectors. Many studies have reported the effectiveness of plant extracts against mosquito larvae. Plant extracts are generally preferred because of their less harmful nature to nontarget organisms, due to their innate biodegradability.

Some of these diseases could in future involve the most productive and strategic plants have rapidly raised in significance and generated great alarm about the consequences of their diffusion. The potential economic negative effects are enormous and the damage to the local living system may be dramatic. Several epidemic emergencies are in action, and the emergency is going to become a normal trend, as a consequence of the permanence of several factors, including in first place climate changes.

On the basis of novel knowledge, some new approaches are emerging, changing the aspect of insect control. Integrated pest management is an important approach, developed in recent years to control disease vectors and limit economic agricultural damage, improving crop yield with minimal cost. Its main goals are to: (a) increase the knowledge of the relationship between insect pests and the other organisms involved, including the target one; (b) reduce pesticide application and quantity developing biological controls, farming practices, farmers' collaboration, and mechanical and physical controls; and (c) build new models of integrated managements on the basis of laboratory and field experiments, including the research of new active compounds. Novel pesticides, to be suitable, must be low cost, eco-friendly, from renewable and sustainable raw materials, nontoxic to nontarget organisms, of rapid degradation, and not accumulate in the environment.

This chapter will be mainly dedicated to the impact of migration by alien species as an element of habitat perturbation. In this case, environmental factors play a fundamental role in the comprehension of the phenomenon and its development. The effect on alien species is based on the potential impact of invasive species on the habitat wherein they are introduced. A habitat can be regarded as a complex dynamic system wherein, under the influences of biotic and abiotic factors, species tend to get their homeostatic equilibrium, and persist in the face of competitors, predators, and diseases. On the basis of knowledge of prior invasion history, emerging alien species can be considered as those never before encountered as alien or those already known but so far considered not invasive. Therefore, the key argument to understand the phenomenon is that the habitat we are considering is alien first for the invader, and that means a series of unsuccessful attempts until a final successful adaptive sequence of steps, determined by some changes in the habitat, which allow the invasion. In other words, the barriers that usually block the invasion can be removed by perturbations changing the habitat. Perturbations due to abiotic and biotic influences include those by the migrating species finding conditions to live even better than where they were native, also as a consequence of important climate changes in action or the possibility of finding food and resources easily. The influence of human activity is an important factor.

In the past, exchange of species between continents was considered an occasion, as demonstrated in the events consequent to the discovery of the New World. Christopher Columbus traveled to unknown lands in search of gold. After three journeys, the quantity of obtained gold was risible, but he found a much more important and economic valuable green treasure. Hundreds of species have been introduced to regions outside their native ranges, from and to America, and many have become permanent additions to local fauna and flora, also favored by agriculture care. Limiting the list to plant cases, examples of this interchange are tomato, cocoa, red pepper, and tobacco on one side and sugarcane, cotton, and mint on the other. In some cases, the introduction was initially complicated by prejudice, such as for potato, due to the general negative attitude against European *Solanaceae*, like henbane, jimsonweed, and deadly nightshade, in consideration of the content in tropane alkaloids and their effects on the CNS. However, American *Solanaceae*, like potato, tomato, sweet, and red pepper, contain alkaloids only in the juvenile stage, and therefore the positive alimentary results overcome any resistance and prejudice. On the other side, the success of cotton and sugarcane cultivations generated heavy impacts on the new habitats, due to monoculture, as well as forced human migration from Africa. One can only imagine nowadays Columbus coming back from a voyage to a new land and facing all the bureaucracy necessary to introduce just one new species from America.

Cultivation and alimentation, and also social equilibria, were radically changed by these introductions, though several other negative aspects, such as the abandonment of native sources of food or medicine, should also be noted. Therefore, devastating changes to local situations were, and often still are, considered to be a necessary secondary effect. Later, this approach changed in favor of maintenance of the current situation, considered the best obtainable. Nowadays, there are severe alerts concerning introduction of alien species, in order to avoid the risks and consequences of their introduction in other habitats and the unexpected damage they can produce. This means that a conservative tendency is becoming dominant in human approaches to the future, although history is full of examples of positive breeding and exchange. The problem is that the immediate effects of a change by introduction must be perturbative, just as in a chemical reaction to obtain a new molecule, it is necessary to break the bonds between the elements of the reagents using the activation energy. The final exergonic energy obtained is the positive result generated by the necessary destructive loss of the original starting point, forced to remain in the past by incoming modernity. Fear about the future, worrying about even minimal changes, before considering all the possible consequences, is a current dominant tendency, and a cause of social and political concern. Any resistance or restoration or attempts, to maintain the obsolete equilibria, are destined to be frustrated or outdated. In any case, besides the obvious initial negative effects, future consequences of an alien introduction are very difficult to predict, as is the assigning of victims and winners. The only smart reaction possible must be based on an understanding of the reasons and causes of the proceeding new order. Again, the analysis of the negative effects on the environment accuses mankind's tendency and exaggeration in influencing and diverting the natural flow of the events. In any case, destruction of the past is a natural consequence of ongoing changes in any single minimal part of the universe.

Exchange of living biomaterials poses a significant challenge to biosecurity interventions worldwide, as demonstrated by several reports and experience in any airport. Alien species can deeply affect current ecosystems, influencing ordinary human health and activities, thereby redefining the classical boundaries of biogeography, although the story of our planet is full of such phenomena, and they can be considered part of the driving forces of evolution and breeding. Invasion of alien species is a consequence of the physical forces governing movements of matter, but the same forces can provoke acceleration or stagnation. In this period, the biodiversity is a phase of rapid change with loss or danger of extinction of a number of species augmenting the extent of migrations.

The research reacted in good accordance to the increase of the alien phenomenon. The number of papers on alien or invasive species published per year (1980–2010), according to ISI Web of Science (accessed July 1, 2011), started from *c.*100 papers in 2000 to reach 1000 in 2005 and 1500 in 2010. The entity of the phenomenon can be deduced by a global database of the first regional records of alien species covering the years 1500–2005. In a database of considered 45,984 species, a screening established a first record of 16,019 established alien species, in order to investigate the temporal dynamics of the occurrence of emerging alien species worldwide. The study evidenced that 1%–16% of all species on Earth, with differences in taxonomic groups, could be qualified as potential alien species and suggested that a high proportion of emerging alien species remain to be encountered, whose future impacts may be negligible or revolutionary. The surprisingly high proportion of species in recent records, which have never been recorded as alien before, means that the potential risk may be high. The high proportion of these emerging alien species mainly resulted from the increased accessibility of new source species pools in the native range. Our ability to predict the identity and impact of future invasive alien species is pivotal, and it is largely dependent upon knowledge of prior invasions and understanding temporal trends, origins, their interactions inside new habitats, and the drivers of their spread, improving prevention and risk assessment tools. Risk assessment approaches that rely on invasion history will need to be prioritized, with the awareness that physical boundaries and governmental declarations cannot halt or prevent biological invasions completely.

Migrations and invasions are a constant phenomenon of biological activity, therefore attempts to control the situations and predict the consequences are probably useless. Despite all controls, the phenomenon is relevant and the rate of emergence of new alien species is still considered high. One-quarter of first records during 2000–2005 were of species that had not been previously recorded anywhere as alien and during the past two centuries, a strong worldwide increase of established alien species was registered.

The current state of the phenomenon shows serious driving forces in favor of an increase of migrations. Although a large variation across taxa was also evident as part of the phenomenon, model results show that the high proportion of emerging alien species cannot be explained solely by increases in well-known drivers, such as the amount of imported commodities from historically important source regions or global effects. Instead, these dynamics reflect the continuous incorporation of possible new regions into the pool of sources of potential alien species, likely as a consequence of expanding trade networks and environmental change. Increases of temperature and climate change, causing land degradation and sources spoilage, are among the prominent driving forces in action and this trend will probably be confirmed in future. Species, so far confined in a traditional territory, are practically forced to move by environment changes. These changes contemporaneously involve the starting territory and the new ones. This process compensates for the depletion of the historically important source species pool through successive invasions, opening the door to new phenomena.

These results suggest the importance of studying carefully selected cases in act. The study of the phenomenon of the migrations in act and their consequences should be released by alerts and any a priori protective reaction, and on the contrary based on the study of dynamics of past invasions and on adequate and better-informed predictions of future trajectories of alien species movements and accumulation. Considering that any alien species was once an emerging species, there must be conditions for its improvement. The dynamics of emerging alien species accumulation can provide a direct measure of ongoing invasion dynamics without the confounding effect of subsequent introductions either from the native range or from already occupied regions in the alien range. Later, some considerations were reported as model cases of potential alien species and the risk in their diffusion in restricted territories. In particular, we considered important the case of "emerging alien species," followed in its delicate transient status to alien species causing interest for environmental impact and diffusion of related diseases.

Invasive alien species

Somewhere else at some time, an emerging alien species can possess a high chance of becoming an alien species arising from a native or already known species. The pool of species involved does not encompass all native species, but is limited to those with a high potential of being introduced and establishing in a new region. Therefore, the flora and fauna of the relevant habitats must be compared. Knowledge about the proportion of emerging alien species will also be important for biosecurity, which often relies on information of known alien species, including species not yet recorded but suspected to have a high risk of arrival and impact.

Again, the past can give us some key information. The Aral Lake was once called the Aral Sea and used to be the fourth largest lake in the world, with an area of 68,000 km^2 and water 16–24 m deep. The original name means "Sea of Islands," referring to the more than 1100 islands that had dotted its waters, but now they are hills emerging from a desert. The Aral Sea drainage basin encompasses Uzbekistan and parts of Tajikistan, Turkmenistan, Kyrgyzstan, Kazakhstan, Afghanistan, and Iran. Until the 1960s, the inflows, consisting of several rivers that fed the Aral Sea, were diverted by Soviet irrigation projects. Since that time, the Aral Sea has declined, the water disappearing and leaving space to the desert. In August 2014, satellite images revealed that the eastern basin of the Aral Sea had completely dried up, and desertification is continuing. The same situation was caused by Mongols, who diverted its two main southern effluents to the Caspian Sea. The eastern basin is now called the Aralkum Desert. Among the sand of the desert, there are still corroded carcasses of boats, once fishing in hundreds on the sea and now destinations for tourists. Most of remaining water is salaried, and the zoocenosis radically changed consequently. The alterations in the biodiversity of the lake represent not only a natural response to a decrease in water level and a subsequent increase in salinity, but also effects of nonnative species introduction. The only organisms able to survive are small crustaceans and microscopic colonies of *Artemia* sp., similar to the *A. salina* that we use in our laboratories to test toxicity. *Artemia* crustaceans were not present until the 1950s; *Artemia* is an alien species since its eggs were introduced by migratory birds. However, fossils reveal that there had been four similar desertifications during the last 20,000 years. Therefore, we have the possibility of a congruence of natural and human causes of the phenomenon, or simply it must be considered the further case of a nonsense action persevered by mankind, to force the natural equilibrium to the own temporary interest. Many similar examples can be considered and analyzed to understand other situations in progress.

Several selected examples of the environmental effects of invasive alien species in different parts of the planet can be reported as case studies. These are focused mainly on native species extinction, causing changes in species richness and abundance, alterations to food web interactions, monitoring of steps of invasion, routes of transfer, economic relevance, social and cultural consequences, etc. The economic impacts usually generate attention and alerts, but in some cases, exotic species have minimal demonstrated impacts in their new environments, or have immediate direct impacts or have indirect impacts that may not be immediately apparent. To distinguish, understand, and consider correctly each case, two aspects can be considered fundamental: environmental changes and the role of native species versus alien ones. So far, there are local cases, interesting limited parts of the planet. They are important case studies to understand the phenomenon and its driving forces.

The first case, dedicated to the Argentine stem weevil, reports on a consolidated classic invasion by an alien species and its catastrophic impact in New Zealand (Barker et al., 1989; Barker and Pottinger, 1986). However, counteractions so far have been unsuccessful. The second study case will focus on a developing insect-borne disease affecting olive trees in a southern region of Italy. Due to its developing and progressive damage, it can be considered a model of this kind of phenomenon. In the third case, fennel and its potential impact in pastures of some USA states are considered.

First case: Impact of alien species over decades

An important and well-reported case concerns the impact of invasive Coleoptera species *Listronotus bonariensis* Kuschel (Argentine stem weevil) to New Zealand natural grassland ecosystems. As already considered, the impacts of changes in isolated territories, such as islands, are very interesting as a case study. In past times, exotic invertebrates were generally considered of low impact on plants in New Zealand's natural ecosystems. This was possibly due to the high level of endemism of New Zealand native plants and their phylogenetic distance from host plants of many invasive plant pests. However, the case of this herbivorous invasive species indicates that geographic and/or taxonomic distances may have limited importance, and in some cases this can result in a clue about the environmental system. The adult of this weevil presents limited dimensions, up to 3 mm long. The body is characterized by a gray color with a waxy covering. This wax plays an important role in camouflage, since soil particles adhere to the wax, and when the insect is stationary, it is difficult to spot. Therefore, the adults, thanks to their mimetic capacities, small size, and ability to remain immobile for long times, are practically impossible to detect in the soil, and virtually invisible to predators or other enemies. Their larvae are tiny, cream-colored grubs with brownish heads, with egg incubation periods of 7–15 days, larval periods of 30 days and pupae periods of 13 days. The presence of the insect is usually obtained through the detection of the eggs, which can be observed in the inferior part of the sheaths, where they accumulate near the soil surface. Eggs present an enlarged form with round apexes. Initially their color is light yellow, but this changes to black and therefore they became visible and can be easily localized on the plant's surface, in particular on leaves. An important part of the battle against pests in agriculture concerns identifying the preference of each species in feeding. Each insect selects plants for feeding, which is part of its behavior in the ecosystem, and often this can suggest strategies for its control. Feeding in the case of herbal species, like cereals (*Graminaceae*) living for a short time, requires an exact correlation between the lifetime of the plant and the growing stages of the insect. Oviposition starts in coincidence with the growing of cereals, like mays and wheat, but damage is produced later, when the larvae are active and the plants are mature and grown. The larva needs large quantity of food to increase rapidly and it feeds insatiably on the crown, the stem apices, and the radicular meristems of the weeds. The eggs hatch and the larvae begin to feed inside the plant stem until they reach the second or third instar, at which point they burrow out of the plant and drop to the ground. Once outside the plant, the larva begins to feed at the base of the turf plant, ingesting plant material from the stems and crown. After the fifth instar is reached, the larva pupates within the upper soil profile and the adult soon emerges. Adults then mate and lay the next generation of eggs over winter to resume the life cycle in the following season. The favored production of eggs causes a massive feeding and the consequent destruction of the host plants, exactly when they should reach their final stage of maturity, just before the harvest. Therefore, the consequence of the presence of this insect is the damage of the crobs, with reduction of the number of plants and loss of production, diminution of the roots' volumes and radical apparatus, until the final complete plant breakdown. It is possible that other aspects are involved, such as the consequent prevalence of some native *Graminaceae* weeds, which pollute the field and probably facilitate pest diffusion.

The species is native to South America, and in particular widely distributed in the central regions of Argentina, Uruguay, Chile, Bolivia, and Patagonia. However, the main damage is reported for Australia and New Zealand. The status as a primary pest in an invaded habitat, not being a notable problem elsewhere in particular in the native regions, is similar to several other species. In this case, Goldson et al. (2001) attributed such eruptions of relatively minor pests to a lack of natural enemies in New Zealand's highly modified pastoral ecosystem. Again, the ability of human activity to change and reshape the territory, destroying natural biodiversity, is the main cause of the emergence of pests. In New Zealand, weeds are affected with consequent serious economic damage in several agriculture sectors and livestock. New Zealand's economy is largely based on highly productive pastoral farming, embracing extensive sheep grazing and large-scale milk production, requiring the availability of enormous quantity of grasses. Grasses are the main pasture for sheep as for other herbivorous creatures, like the Argentine weevil. Pasture covers more than 10 million hectares (38%) of New Zealand

and is the largest single land use in the country. This is made possible by a temperate climate and large territories, modified with heavy investment in land improvement, including the introduction of European grasses and regular application of imported fertilizers. Highly skilled farm management by owner-occupiers constitutes one of the highest ratios of capital to labor in farming anywhere in the world. It is important to consider that lamb, mutton, and beef represent a fundamental part of New Zealand's economy, including their globally precious wool. Wool was New Zealand's main export earner from the 1850s until the start of the 20th century. In 1920, wool contributed 26% of New Zealand's total value of exports, but by 2011 its contribution had fallen to 1.6%.

However, we must always remember that human activities are interconnected, as well as the consequences, and therefore we must refer to history and to the most impactful episodes, like wars. The New Zealand wool boom, which started in 1951, was a direct consequence of the U.S. policy in the Korean war (1950–1953). At the beginning of the war, the USA sought to buy large quantities of wool, necessary for soldier uniforms and to maintain stockpiles. As soon as the New Zealand wool was chosen, the prices tripled overnight and farms were asked for record production. After the war ended, the export price of wool declined, until it fell to 40% in 1960. However, the biological machine had already been started and New Zealand's sheep population continued to rise and produce. The sheep numbers rose from 34.8 million in 1951 to 70.5 million in 1982. A series of measures by the government caused a decline of this number, until it reached 39.3 million in 2004, and a slight increment of the sheep population put it at 40.1 million in 2006.

Nowadays, New Zealand is the world's top dairy exporter, accounting for a third of the world's dairy trade. Dairy alone now accounts for 35% of New Zealand's total commodity export value. Total sheep numbers as of June 30, 2018 totaled 27.3 million. However, this is down 0.8% on the previous June, whereas total beef cattle numbers on June 30, 2018 totaled 3.68 million, up 1.9% from the year before. Furthermore, total dairy cattle numbers rose slightly to 6.60 million at June 30, 2018 due to a decrease in the numbers of cows and heifers. Total beef and veal receipts were expected to total $3.42 billion FOB in 2018–2019. However, New Zealand beef and veal exports were forecast to decrease by 3.1% to 415,000 t shipped weight, despite the more favorable exchange rate. For 2018–2019, the number of cattle processed for export was forecast to decline by 3.0% to 2.51 million. This forecast decrease followed from a 9.6% increase in

2017–2018, due to a large increase of cows and bulls. The mix of cattle classes was expected to remain relatively steady in 2018–2019; however, some alien species have been causing a decline of production, including the famous Merino wool.

Let us now return to the alien species with the last part of this story. The high quality and production of products derived from pasture fueled a high rate of export of meat and wool, until the invasion of New Zealand's native grassland flora by the Argentine stem weevil. The presence of this species in New Zealand was first reported in late 1927 by Marshall (1937). However, its introduction is likely to have been earlier in the 20th century and there may have been more than one introduction. In any case, in the next 30 years the occurrence of this weevil became an abundant and massive agricultural pest. It was first recognized as a pest to wheat in 1933, but it was not until the late 1950s that the weevil's potential for damaging pasture was recognized. From that period, the impact of this pest increased and major damage was evidenced in crop grasses, and in some of New Zealand's introduced Graminaceae including common cereals and pasture grasses, like perennial ryegrass (*Lotium perenne*) and Italian ryegrass (*Lotium multiflorum*). *L. bonariensis* is also a major pest of cool climate turf grasses in Australia, and can frequently be found in association with these plants. The consequence is that this weevil has been highly adventive and has acquired host plants that it did not evolve with, at least at a species level. This is the main reason for its diffusion and consequent agricultural damages, which however were not equal in whole New Zealand.

Several other exotic species of Curculionidae have been studied in New Zealand native grasslands, but few have been recorded to be feeding or breeding on New Zealand native plants. Their presence in native grasslands may often be simply a case of vagrancy, for example, in the case of the lucerne weevil, *Sitona discoideus* Gyllenhal, but its hosts' preferences are restricted to species of *Medicago* spp. and *Trifolium* spp., and it is unlikely to have host plants in New Zealand's native flora. In contrast, a flightless, polyphagous, European weevil, *Otiorhynchus ovatus* L., which occurs in tussock grasslands in central Otago, might feed on some New Zealand native plants. Furthermore, this species, and three other *Otiorhynchus* spp. that are established in New Zealand, were not recorded on native plants sampled. That means that each case must be considered and studied as singular, and general considerations or extrapolations may be misleading.

Having become established throughout New Zealand, by the early 1990s *L. bonariensis* was estimated to be causing damage to the intensive pastoral sector amounting to NZ$78–251 million (US$50–170 million) annually. Responses were focused on the use of pesticides and biological control, and have been considerably offset by the successful introduction of the parasitoid *Microctonus hyperodae* Loan (Hymenoptera: Braconidae) in 1992, but recent research has indicated that *M. hyperodae* is becoming less effective as a biological control agent for reasons that are not entirely understood. This is a further indication of the difficulty of biological control to produce the expected results, or probably that the natural clock needs different times for production of visible effects.

Second case: Novel insect-borne disease in action

The second case was selected due to its potential impact. Therefore, in this case, we shall try to understand the conditions for development of a dangerous alien species.

We shall focus on an insect-borne disease, though the scenario is far larger. Pathogens are distributed everywhere and their targets are any organic matter, including living organisms. Their effects on cultivated plants are under examination, with some cases of particular interest, with insect-borne diseases first in line. The interest is strictly related to the economic damage. Therefore, there is no real interest in environmental impact; effects on crops and cultivated plants are only considered for the loss of production.

We must recall that insect-borne diseases are the results of a complex multiorganism interaction. The network of several different collaborating organisms is on the basis of diffusion, effectiveness, and metabolism of insect vectors, including the resistance phenomenon. The integrated network acts like a “superorganism,” integrating functions of all the different types of involved organisms. A disease affecting the host is the result of a determined and useful collaboration between totally different organisms, from bacteria to multicellular organisms, and an integrated system is key to survival and proliferation. This is an important lesson, based on the consideration of the existence of several levels of eco-friendly interactions in the environment. The consequences, which we consider as negative, are only the collateral effects of competitive struggle. Here, an important recent episode of insect-borne disease affecting olive trees is reported (Figs. 5.20 and 5.21). In addition to the economic impact, the Xylella affair is an emblematic case evidencing the difficulties of facing such emergencies with ordinary responses (Benelli, 2018; EPPO/OEPP, 2016; Nicoletti et al., 2016; Purcell, 1997).

Fig. 5.20 The effects of Xylella attacks on olive trees in Apulia.

Fig. 5.21 All the olive trees in this field were devastated by Xylella.

- *Xylella fastidiosa* and the olive destiny
- Chronology of *Xylella fastidiosa* outbreaks

1870: Reports in California of grape wine "mysterious disease" causing the deaths of plants.

1890: The disease practically disappeared or was not recorded.

1892: Newton B. Pierce reports on the disease in California and on the damage to grape plants.

1920: New epidemic diseases appeared in California, apparently not linked to the previous episode.

1920: Alfa-alfa disease (AD), no other cases reported.

1930: Hewitt names the rediscovered grape wine disease the Pierce disaster (PD).

1930: Reports on disease in peach and other trees.

1933: PD spreads in southern USA and is considered relevant from an economic point of view.

1940: Major epidemic disaster; vectors come from alpha-alpha (AD) through a "virus"; the research is related to xylem sap-feeders and considered xylem-limited.

1970: Almonds and oaks also affected; symptomless plant host discovered.

1972: PD is considered important, but classified as one of several diseases affecting grape wine and other cultivated species.

2011: First cases of dehydrated olive trees near Lecce town in Puglia; the disease is named Olive Quick Decline Syndrome (OQDS) on the basis of its effects.

2012–2014: During these years, in most of Salento, which is the peninsula of Apulia region, the presence of *Xylella* spread affecting the centenary olive trees. The analyses report an increasing presence of OQDS.

2014: The disease spreads, affecting more than 9000 ha. The alert is raising public concern as well as farmers' warnings.

2014: An International Symposium on the European outbreak of *Xylella fastidiosa* in olive trees is held in Gallipoli (October 21–22, 2014), and followed by technical laboratory workshops at the CRSFA, Locorotondo (October 23–24, 2014). This was the first meeting about OQDS, with the participation of more than 200 experts. The Proceedings of the Symposium are published in the *Journal of Plant Pathology* (2014), 96 (4, Supplement).

2015: The counteractions to limit OQDS demonstrate inefficacy; the only adopted measure is the eradication campaign, in accordance with EU protocols and financial supports. Eradication of the infected trees starts with the destruction of dozens of trees, and continues with the removal of any plants near the affected trees. The population of the region demonstrates against the eradication campaign.

2015: The regional court accepts the considerations against the eradication, but the campaign goes on although slowing down respect to the original plan. In July, the French authorities notify the Commission of the first outbreak of *X. fastidiosa* subspecies *multiplex* in Corsica and in PACA (departments: Var and Alpes-Maritimes), 25 outbreaks in PACA and some 350 in Corsica. The presence of *X. fastidiosa* subsp. *pauca* is also detected in one outbreak in PACA (Menton). Ornamental plants, such as *Polygala myrtifolia*, cause the main host plants to be affected, although new plant species are detected as investigations progress.

2016: A line of containment on the northern boundary of Salento is adopted. The line consists in 2km of eradication of any plant, situated coast to coast between the Ionic Sea and the Adriatic Sea.

2016: Sporadic news of the disease's presence is reported in Corsica and other parts of Europe.

2017: By the beginning of the disease up to 2017, Xylella has infected up to 1 million trees, causing the death of most of them. Reports about the presence of disease outside of the containment zone are presented, whereas in Salento the massive ecological disaster is evident.

2018: In March, the Italian authorities notify the presence of *Xylella* in different parts of the buffer zones already established with a large number of outbreaks in the zone adjacent to the northern zones of the buffer zone. On June 27, the EU Commission extends the demarcated area in Apulia by around 20km toward the north of the region. The EU Commission starts a campaign of controls about Xylella to entire territories of state members (EPPO/OEPP, 2004). In 2016–18, 40,600 inspections on 20,000 samples were analyzed in garden centers, nurseries, and other sites across the EU territory, with the exclusion of the demarcated areas. In the demarcated areas established in the EU territory, instead, more than 110,000 samples were analyzed in 2016. On April 10, the Spanish authorities also notify the Commission of the first presence of *X. fastidiosa* susp. *Multiplex* on one olive plant in an open field, in Spain mainland, in the autonomous region of Madrid. The area is demarcated and eradication measures taken, and movement of specified plants out of that area is blocked.

2019: The number of continuous outbreaks leads to the conclusion that eradication of the pest in the buffer zone is no longer possible and, because of the significant delays in the removal of those infected plants, the risk of further spread toward the north of the Apulia region is possible, since the range of host plants increases as investigations progress. In Apulia, Xylella has mainly infected olive trees. No infections have been confirmed so far on *Vitis* and *Citrus*. Movement of specified plants out of demarcated area is currently not authorized, except for grapevine nursery material subject to hot water treatment. Olive cultivars resistant to Xylella are started in several areas of Salento where trees had been eradicated. As *X. fastidiosa* is considered to be established, the entire territory of Corsica is declared as an area under containment. At the present time, the EU territory, with the exception of the officially demarcated areas, is considered free from *X. fastidiosa* based on official surveys.

2019: Several analyses report and confirm the presence of Xylella out of the zone so far considered infected, causing a general alert about the expansion of the disease.

2019: OQDS is currently considered as one of the greatest threats to European agriculture and landscape, with economically relevant damage and difficulties in stopping its further diffusion. Olive oil producers in Apulia react to the OQDS damage and consequent decrease of olive oil production with massive imports of oil from Spain and Greece. The different quality and cost of the imported oil cause an invasion of low-cost oil in the market.

The second case here reported concerns the olive tree and the production of olive oil in a region of Italy, once the most productive. Olive tree (*Olea europea* L.) is a wood species endemic of Mediterranean regions, wherein Greece, Spain, and Italy in particular are contending for primacy in olive oil production. The olive tree has been cultivated from ancient times and was probably one of the first plants to be selected for production of edible oil of high quality using olive oil mill (Fig. 5.22) totally dedicated only to this kind of product. Although the species is highly resistant and able to live for more than a century without particular treatments, recently a great alert changed the scenario completely and the very survival of most olive trees is in danger. The current numbers evidence a situation that cannot be underestimated. In 2018, the Italian production of olive oil registered a decrease of more than 43% in comparison to 2017 (185,000 t against 428,922 t). The Italian production was inferior to that of Greece, which also reported a decrease. Spain confirmed its first place with about 1.5 million tonnes, with an increase of 24%. However, the Spanish production is the result of enormous imports from Northern Africa and of production methods with

Fig. 5.22 The modern system of extraction of olive oil in Italy, useful to obtain extra virgin olive oil of high quality.

devices that are not utilized in Italy. The cost is lowered, but the quality cannot be compared. In practices, the trade name "extra virgin" is the same but the oils are very different. The important nutritional properties of olive oil, as a fundamental ingredient of the famous Mediterranean diet, are at risk, as well as the presence of precious antioxidant polyphenols and unsaponifiable oily compounds, but there is much more. In Italy, food like olive oil means tradition and culture, including a great variety of produced oils. Each region is proud of many cultivars selected from a long time ago and people consider as part of its character the taste and the quality obtained with care and traditional methods. The same goes for wine, pasta, cheese, and many other food products, appreciated worldwide for their unique characters. Traditional and distinct foods are considered a necessary mark for each town and village. Food made in Italy, synonymous with high quality and precious tastes, is made by a mixture of multiple differences and highly variable local production, generated by the fusion of ethnical different traditions both near and far, in part autochthonous and in part consequences of the influences of many invasions affecting the Italian peninsula and its main islands. Italian food products are desired by many producers in the world, but their quality cannot be reproduced.

In 2017, the per capita consumption of the olive oil in Italy was 8.9 kg yearly (+4.7% vs 2017), but the same year registered also an increase of the prize up to 40%. The reasons for these changes in a single year were considered a consequence of the weather in the 2017 winter, which had an exceptional freezing week, affecting seriously the olive trees of several regions, but in one part of south Italy the problems were very different and related to an insect-borne disease.

Why in Apulia?

First, why in Apulia? Italy is a peninsula, whose geographic form is considered similar to a shoot, or more often a boot. Islands and peninsulas are very interesting habitats, because of their endemisms, caused by their isolation, and capacity to preserve and produce living varieties. Apulia is the heel of the boot, and therefore a peninsula of a peninsula. Its territory pushes out into the Adriatic Sea, in great part virtually isolated from the rest of Italy. It is a plane territory, mainly surrendered by the Adriatic Sea, in the ideal position for the exchange with the Orient, characterized in the past by the import of species and silk from the Orient and the export of food of high quality (Fig. 5.23).

Before any consideration about the recent disease that destroyed the olive trees in this part of Italy, named Salento, we must consider the environmental factors and the sequence of events already reported. Salento is a sub-peninsula of the Italian peninsula, full of history. Because of its position and its arc form, it is described as the "heel" of the Italian "boot." The peninsula is also known as "Terra d'Otranto" and in the past was named Salentina. In ancient times, it was known as Messapia, because during the Bronze Age the Salento peninsula was inhabited by Indo-European populations. In fact, the first reports about the inhabitants of this land, around the 5th century BCE, concern the Messaps population, who were dedicated to agriculture, horse breeding, and pottery. Testimonies of this period are the 10 dolmens and menhirs in the lower part of the land, as well as the construction of cities with imposing walls. Salento is in the middle of the Mediterranean Sea and, being in front of Greece, looks like a bridge between central continental Europe and the Orient.

Fig. 5.23 The particular geographical configuration of the Apulian peninsula.

Due to its strategic position, Salento was the theater of several civilizations. Later, Salento history met with Oriental history due to its Mediterranean facing, and according to legend, inhabitants of Crete founded the town Lecce. In fact, during the 8th century, Greek settlers founded along the coast several cities that are still very important, such as Gallipoli, Otranto, and Taranto, which would become landmarks of Magna Grecia, becoming small capital cities more prestigious than those of the fatherland. Romans and later Byzantines dominated the region, until the Norman rule that, with Federico II, brought the region to be a very important cultural center. Under the Swabians, a long period of decadence started and the peninsula went through a long series of invasions, sacks, and destruction. Another dramatic period for Salento was the Turks' invasion that in 1480 attacked and sacked Otranto, whose resistance was punished by the killing of 800 inhabitants.

Under Spanish rule, starting from the 16th century, towns were fortified and Lecce became one of the most beautiful and important cities for cultural and artistic activities. The beauty of the inland parts with their baroque aspect attracted nobles and scholars, and nowadays the magnificent architecture is considered unequaled in the world. The beautiful buildings of that period, known as the "Apulia's Baroque," are made by stones of blank color, typical of the territory.

All these civilizations sacred the olive trees and the olive oil, which from antiquity is extracted from the drupes with sapient art. Salento's landscape is dominated by magnificent olive trees, which state as silent giants stating from centuries to protect the territory and signing the boundaries between the blue of the sea, the gold of the coastal sands, and the green of the vegetation. The habitat, dependent on the olive trees for thousands of years, without their protection now risks desertification as a possible future.

In 2013, this beautiful part of south Italy, the Salento Peninsula, well-known for its production of olive oil and wines, was troubled by a dramatic phenomenon, never reported in human memory. The olive trees started to lose their leaves and were rapidly reduced to skeletons. It all started somewhere near the town of Gallipoli, in the south of Apulia. The diffusion of the decline was rapid and in April 2015 the whole province of Lecce and other zones of Apulia were highly affected, always focused on the Salento Peninsula. Almond and oleander plants in the region also tested positive for the same disease, but without epidemic episodes. The disease has been called Olive Quick Decline Syndrome (OQDS) and its characteristics had never been recorded, in particular on olive trees.

The disease first caused withering and desiccation of terminal and lateral shoots, which are usually produced by the tree in great quantities and distributed randomly. OQDS results in the collapse of the rest of the canopy and ultimately the death of the trees. The giant trees, considered an emblem of nature's puissance and vitality, which survived for centuries despite any kind of offense and difficulties, in a few months were reduced to pitiful skeletons. The scale of the phenomenon was underestimated and information largely lacking until, at the beginning of 2015, the epidemic feature of the disease was evident to everyone, with thousands of centenary olive trees completely dehydrated by the disease. Up to a million olive trees were clearly affected or resulted infected in the southern part of Apulia. Only two evident responses were performed: a careful detection of the diffusion of the disease, and the eradication of affected trees and the nearest ones to these trees. After the eradication, the treated areas appeared totally changed, practically like a desert. In addition to the heavy economic damage, loss of the olive trees means a tremendous cultural and environmental impact on this territory, where they are the symbol of region's identity and provide important support for tourism. Nowadays, the situation reports damage totaling 1.2 billion euros and 10 million plants damaged in a territory of 720,000 ha, practically most of the Apulia territory. This could be only the beginning.

The disease

The bacterium responsible for the disease is considered to be *Xylella fastidiosa*. In this regard, OQDS arose due to a well-known plant disease, which affects several economically important plants including grapevines, almond, pear, peach, coffee, and citrus, but ornamental plants such as oleander and *Prunus* spp., forestry crops (sycamore, mulberry, red maple, elm, oak), several weeds, and native plants are also infected (Romi, 2010; Reiter, 1998, 2001). In the latter cases, the bacterium often does not cause any visible symptoms, but the plants act as efficient healthy carriers. The disease is called PD (Pierce's disease, or even PD disaster), from the name of the scientist who first reported the effects. However, although the presence of *X. fastidiosa* in Italy had already been reported, OQDS was totally unexpected, although the conditions for the infection had already been reported by scientists. Plants infected by *X. fastidiosa* mostly grow in parts of the American continent (North, Central, and South America), that have mild winters and long growing seasons, in particular in southern California, whose climate is similar to that of the Mediterranean (Chatterjee et al., 2008). The disease models have long predicted that the pathogen could establish in Mediterranean regions, owing to similar climatic and environmental conditions. For instance, Greece, southern Spain, and Italy have been identified as particularly at risk from *X. fastidiosa* if vectors of the bacterium occur.

Indeed, in southern California, both the host range and spread of *X. fastidiosa* have expanded further as a consequence of the introduction and spread of an alien new vector, the glassy-winged sharpshooter (*Homalodisca vitripennis*). Wong et al. (2005) from the University of California-Riverside reported olives as a host of *X. fastidiosa*, examining more than 500 samples from plants located in five cities (Fillmore, San Diego, Redlands, Riverside, and Tustin) in southern California counties. After increasing incidences of olive tree mortality occurring in the Los Angeles area in 2008, surveys were conducted by Krugner et al. (2014) to evaluate the association of *X. fastidiosa* with scorch and dieback symptoms in olive trees in southern California and the southern San Joaquin Valley. However, until the arise of ODDS, no significant alert was reported, and *X. fastidios*a was considered among the 150 pests affecting olive trees (Janse and Obradovic, 2010; Krugner et al., 2014). In fact, PD had previously been considered to affect mainly grape wine and sporadically other plants, such as oleander, almond, cherry tree, *Polygala myrtiflora*, *Spartium junceum*, and a high number of other targets, with different effects (Costa et al., 2000). PD is known to be prevalent within the USA from Florida to California and outside the USA in Central and South America, with varying effects and impacts (Hernandez-Martinez et al., 2006, 2007).

Therefore, this is more or less the story of a unique phenomenon, whose nature and causes must be considered with great attention, at least for the dramatic consequences and the possible scenario. Owing to the climate and soil condition, as well as a strong tradition of cultivation, olive trees are the dominant plants in Apulia, real living monuments to the spirit of this territory. Cultivation of olive trees has been a fundamental occupation of the local population as well as a key component of alimentation. In 2015, the olive oil produced in Apulia accounted for 60.4% of total Italian production, followed by Calabria and Sicily with 17% each. In 2018, oil production in Apulia decreased by 58%, with damage costing 1 billion euros. To face the increasing requirements of the Italian market, the result was an import of 43.3 million euros' worth of oil from Greece and Tunisia. The imported oil was converted and marketed as Italian oil, despite its low quality, and caused confusion and disappointment in consumers. However, if adverse weather conditions are considered a normal possibility and producers have faith in the next year, the ODQS disease may be a different matter entirely. People, and especially the scientific community, were totally unprepared to face the situation and the first response (or lack of one) was to wait for a natural positive solution by the capacity of olive trees to solve the problem independently. Even information supplied to the national population and press was limited until the damage from the disease was too evident. The first data of 2019 registered a decrease in the local production in the region, but a great quantity of olive oil in the market at low prices. A liter of extra virgin olive would usually cost at least 8–10 euros, but in the supermarkets it was going for as little as 2–3 euros per liter. This olive oil was imported and very different in composition from what was locally produced. The consequence is that in Apulia, oil companies have plenty of stocked imported oil, ready to introduce into the market as locally produced, thanks to the absence of any legal protection by the EU.

Once again, the correct interpretation of a vector-borne disease must rely on the past. As already reported, roots of ODQS can be found in the previous story of the similar disease, PD. Everything started unexpectedly in the 1880s, when a "mysterious and unknown" disease spread in the Los Angeles area in California, affecting deeply the precious cultivation of grapes (*Vitis* spp.). Rapidly, the new disease assumed an epidemic state and destroyed *c.*14,000 ha of grapes. Approximately 50 wineries had to close down because of shortage of materials. In 1887, N.B. Pierce (1856–1916) studied in detail the disease and reported the diffusion methods and the affected plants. He described the disease on grapes in California near Anaheim, and therefore it was known as "Anaheim disease." Later, the disease was named after him: Pierce's disease (PD) of grapevines. Growers, administrators, policy makers, and researchers worked hard on finding a solution, but no cure has been identified. However, the research was successful in identifying the Gram-negative bacterium *Xylella fastidiosa* as responsible for the disease. The mechanism of the pest was explained by the introduction on the scene of the vector, identified as a glassy-winged sharpshooter. A related disease was recorded in peach (*Prunus persica*) in 1890 in the USA, with outbreaks (mainly in Georgia) in 1929, 1951, and 1976; this was named phony peach disease (PPD). The causal agent of PD was isolated from grapes in a pure culture for the first time in 1978 (Davis et al., 1978, Turner and Pollard, 1955, 1959). Now, 125 years later, PD is still a significant concern for grape and wine producers in the southern USA (especially California, Texas, and Florida), and the affected plants account for 150 species and innumerable cultivars. Since 2013, the centenary olive trees, considered the heart of the natural landscape of the region, started to die. The cause was deemed to be a vector-borne disease, known as PD, although with several differences compared to past episodes (Almeida et al., 2005). The bacterium responsible was *X. fastidiosa*.

In Italy, the disease's progress is in accordance with the epidemic model. Everything started from a little area in Gallipoli, near the town Lecce, practically at the end of the peninsula. Slowly expanding in the first year and during the subsequent 2 years, most of the olive trees of the southern part of the Apulia region were totally destroyed. Thereafter, the diffusion was very rapid, epidemic, and devastating. Concerning olive trees, PD had previously been considered one of 100 diseases affecting the species, without any report of epidemic virulence. The disease menaces the surviving olive trees, at least in southern Italy. Therefore, affected countries, like France and Greece, are on alert and asking for rapid control of the disease before pandemic diffusion.

The first key consideration is that such virulence of a PD disease never experienced before presented several relevant coexisting novelties, concerning the vector and the parasite. Therefore, traditional methods of control must be considered obsolete or inadequate. New problems ask for new solutions and other points of view.

The parasite

Preferred common name:

Known as the pathogen of Pierce's disease of grapevines, which is spread by xylem feeding leafhoppers, known as sharpshooters.

International common names:

English names: alfalfa dwarf; almond leaf scorch; citrus variegated chlorosis; dwarf lucerne; leaf scorch disease; oleander leaf scorch; pear leaf scorch; pecan fungal leaf scorch; pecan leaf scorch; periwinkle wilt; phony disease of peach; plum leaf scald (Table 5.7).

Table 5.7 Scientific classification of the parasite.

Domain	Bacteria
Kingdom	Eubacteria
Phylum	Proteobacteria
Class	Gammaproteobacteria
Order	Xanthomodales
Family	Xanthomodaceae
Genus	*Xylella*
Species	*Xylella fastidiosa* (Wells et al., 1987)

The pathogen of ODQS disease was precisely identified as *Xylella fastidiosa* subsp. *pauca*, strain CoDiRo. *X. fastidiosa* is an aerobic, xylem-inhabiting, vector transmitted, Gram-negative bacterium of the monophyletic genus *Xylella*. The microorganism form is typically rod-shaped, with a diameter of 0.2–0.5 μm and a length of 1–4 μm. Their cells are covered by a thick cell wall, with grooves and ripples. There are several intrinsic problems when studying and trying to control Xylella. The name *fastidiosa* was assigned in consideration of the difficulty to culture the causal agent of PD on an artificial medium, and in any case very slow growing organisms can be obtained. The attempts to reveal the parasite by Pierce stated in the 1880s, but the causal agent of PD remained a mystery for long time. It is noteworthy in the above classification of the parasite in 1987, indicating the relatively recent scientific determination of the bacterium. In fact, for a long time the microorganism was able to dodge the analysis of scientists, many of whom considered that it could not be cultured at all outside the host and was generally presumed to be a virus or a nonculturable bacterium. In only a few cases was it possible to culture the bacterium successfully on an appropriate agar media. Now, considering that cultivation in laboratory conditions is still practically too difficult, genomic methods of detection must be used. Again, we are facing a galaxy of organisms, complicated by any other microorganism modulated by the need of adherence to the microhabitat. *X. fastidiosa* can be divided into four subspecies (and several strains) that affect different plants and have separate origins. *X. fastidiosa* subsp. *fastidiosa* is the most studied subspecies, as it is the causal agent of PD; it is thought to have originated in southern regions of Central America and is able to affect a great number of different plants. *X. fastidiosa* subsp. *multiplex* is thought to originate in temperate and southern areas of North America and can affect many trees, including stone fruits such as peaches and plums. In particular, *X. fastidiosa* subsp. *pauca* is thought to originate in South America and can affect South American coffee crops in the form of coffee leaf scorch. *X. fastidiosa* subsp. *sandyi* is believed to originate in the southern part of the USA and cause oleander leaf scorch disease. The subspecies causing PD in Salento is *X. fastidiosa* subsp. *pauca*, and this was a source of speculation about its introduction in Salento.

X. fastidiosa, once installed, infects the conduction system of the host plant. The proliferation of the parasite works by blocking the xylem, which conducts the water and minerals around the plant. Within susceptible plant hosts, *X. fastidiosa* forms a biofilm-like layer within xylem cells and tracheary elements that can completely block the water transport in affected vessels.

Symptoms include chlorosis and scorching of leaves, and the entire plant can die after 1–5 years. Unusual features of the bacterium virulence in Salento were the speed and scale of the damage to olive trees. Pierce's disease is considered less prevalent where winter temperatures are cold—that is, at high altitudes and in inland northern areas. In these conditions, the bacteria are not in a simple situation. They do not have locomotion organs, like flagella, and, therefore have limited active mobility. However, at least one subspecies has two types of pili of different size and on only one pole; longer pili are used for locomotion while shorter pili assist in biofilm formation inside their hosts. These details are important since the bacterium needs to move with a characteristic twitching motion, traveling upstream against the heavy flow of the sap inside the xylem vessels. The bacterium has a two-part life cycle: inside an insect vector, and inside a susceptible plant. While the bacterium has been found across the globe, only once the bacterium reaches systemic levels do symptoms present themselves.

The vector

The vector question is very important although so far underestimated, since among the hypothesis of OQDS explosion, it is possible that *X. fastidiosa* acted as a quarantine agent in Europe that had been previously reported in the Mediterranean region, but did not spread, probably because of the lack of a suitable and efficient vector (Table 5.8). The vector could be an alien species, which found a suitable environment, or an already present insect, induced to change its plant host. In any case, the climate and habitat change effects can be key factors.

Table 5.8 Classification.

Kingdom	Animalia (animals)
Phylum	Arthropoda (arthropods)
Subphylum	Hexapoda (hexapods)
Class	Insecta (insects)
Order	Hemiptera (true bugs, cicadas, hoppers, aphids, and allies)
Suborder	Auchenorrhyncha (free-living hemipterans)
Superfamily	Cercopoidea (spittlebugs)
Family	Aphrophoridae (spittlebugs)
Genus	*Philaenus*
Species	*Philaenus spumarius* (meadow spittlebug) (Linnaeus 1758)

Several vectors of *X. fastidiosa* have been proposed in the case of Pierce's disease, in accordance with insects present in the affected territory. Among them is the glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar), which is a large leafhopper, species native to the Southeastern United States. It is an alien species introduced into southern California, where it has become a serious threat to viticulture. Glassy-winged sharpshooters have large smoky-brown wings with red markings and are very good flyers, making them able to transmit plant diseases further than other vector leafhoppers, such as the blue-green leafhopper, *Graphocephala atropunctata* (Signoret).

Xylella is an obligatory insect vector, transmitted by the bite of xylem-feeding insect feeding into the xylem of a host plant, but infected plant material for vegetative propagation (i.e., grafting) can produce mature plants, that also have the same disease. In the wild, infections tend to occur during warmer seasons, when insect vector populations are at their highest.

The bacterium relies on insect vectors. Known vectors of *X. fastidiosa* are xylem-sap feeder insects belonging to the families Cicadellidae, Aphrorophoridae, Cercopidae, and Cicadidae within the Cicadomorpha order, generally known as spittlebugs. The latter are named due to their ability to produce a large quantity of spittle in the form of a white foam. The insect feeds on the xylem, the water-conducting tissue of both herbaceous and woody plants. Thanks to the xylematic conductor system, the water and mineral salts contained in the soil can reach the active photosynthesizing tissues, mainly present in the leaves. The known vegetal host range of the bacterium is vast, including more than 100 plant species (Turner and Pollard, 1955, 1959). Preferred plants depend on the season and locality, but, in general, the preferred species include crape myrtle, citrus, and holly. Glassy-winged sharpshooters tend to feed on last year's growth and meristematic growth, and excrete copious amounts of liquid as they feed. They ingest 100–300 times their dry body weight in xylem fluid per day, and in large populations, their high volume of excreta ("leafhopper rain") can become a problem, leaving white residue on leaves. Due to the dilute nutrient content of xylem fluid, glassy-winged sharpshooters must have special adaptations to obtain the proper balance of nutrients. Feeding times are thus orchestrated to coincide with the period of peak nutrient content in the host plant. In addition, a specialized structure of the digestive system known as the filter chamber is necessary to recycle the ingested fluid and improve nutrient absorption (Fig. 5.24). The final resulting excreta, as expected, is 99.9% water, inorganic ions, and ammonia, the sharpshooter's excretory form of nitrogen.

Fig. 5.24 Typical foam produced by the vector.

If there is no secretion, you can look to the movement of the adults. These insects are also known as froghoppers, and this name derives from both the resemblance of their body shape to that of a frog and their prodigious jumping ability. If you are near a plant with this kind of insect, you may see them jumping 1 m or more in every direction, despite their tiny size of 5–7 mm. *Philaenus spumarius* holds the world record for insect jumps. When leaping at an angle of 58 degrees above the horizontal, some have reached a maximum height of 58.7 cm above the ground. This means that this insect is able to move efficiently also vertically; starting from the ground level of herbaceous plants, it can easily reach the high level of trees.

If you have entomologic experience, you may notice two tiny black spots at the tip of the head and raised veins in the wings in the adults, whereas nymphs are green with black antennae. Bites of the adult cause discoloration and formation of the attacked plants caused by nutrition, but the insect did not represent a serious cause of depreciation or loss of agricultural production until *P. spumarius* was identified as the main vector of *Xylella fastidiosa*, one of the most dangerous bacterial plant pathogens worldwide and considered responsible for ODQS (Carlucci et al., 2013). The meadow spittlebug, *P. spumarius*, is one of the most abundant field insects in the Apulia region, although other species are probably involved (Rodriguez et al., 2019). The spittlebug feeding on the xylem-sap possesses a piercing-sucking beak, named a rostrum. Digging the rostrum into the tree for feeding, the insect causes the infection and the bacterium propagation closes the xylem vessels, causing dehydration of the plant. Therefore, once introduced the bacterium is diffused inside the plant by the xylem transport system. During this transportation, some populations of the bacterium proliferate inside the vessel, causing occlusion and blockage of the lymph sap and a drastic deficiency of water in plant tissues.

Leafhoppers are medium-sized plant-sucking insects comprising around 2400 species worldwide. They tolerate a wide variety of hosts and are associated with nitrogen-fixing legumes, actinorhizal plants, and some other plants which have a high xylem amino acid concentration. The meadow spittlebug is a homopteran insect very successful due to its polymorphism and adaptive capacity (Dongiovanni et al., 2018, 2019). Therefore, it is probably one of the extensively studied species in ecology and genetics, since it was studied with great attention by biologists for decades, fascinated by all these natural deviations from a reference model. Adults exhibit a heritable mimetic changes of color and pattern on the dorsal surface throughout its range, which also occurs in certain ventral parts. Another interesting aspect concerns its polyandrous nature, since females may mate several times with different males, meaning that the offspring of a single female may be fathered by several males. The result of an increased heterogeneity and high fitness is the present of the vector in habitats of great diversity, and therefore a potential of wide global distribution. However, probably the most important character is that this insect is highly polyphagous, since it feeds on wild herbaceous and woody plants, including some species of agrarian interest, accounting for more than 400 species, and reports mainly concern damage to alfalfa, red clover, wheat, oats, corn, and strawberries. However, some researchers have already noted relationships between spittlebugs and exotic plant species, in studies concerning the influence of alien species in evaluating whether introduced plants favor native or exotic and generalist or specialist herbivorous insects in newly evolving plant-herbivore networks. The results suggest a careful interaction between the alien species and the invaded habitat, wherein plants, insects, and other organisms must be included. Therefore, generalist herbivores can be favored by the invasion of the introduced plants. In contrast, exotic insects can be supported by introduced plants, increasing the local occurrence and range of insect pests. Herbivore accumulation on invasive alien plants increases the distribution range of generalist herbivorous insects and supports proliferation of nonnative insect pests.

If you want to feel the presence of the meadow spittlebug, you can take a walk in late summer and these little insects, when disturbed, will jump everywhere. Otherwise, in early summer you may see them at the stems of herbaceous plants. In summer, females deposit up to 400 eggs on the bark of the plant. In springtime, the eggs open and release the larvae which rapidly move to the nearest herbaceous plants. Later, the larva feeds on the stem of the host plant, and it is specialized in feeding on the lymph inside the tracheid vessels. The xylematic sap is low in nutrients, being constituted mainly of water and minerals. Therefore, the nymphs are forced to ingest large quantities of the sap, and their presence can be detected by the typical lather caused by the sticky foam, resembling the guttation in some plants (*spumarius* means "frothy"). The larva secretes this froth, known colloquially in different countries as "cuckoo-," "witches-," or "frog-spit." The foam is thus no more than the waste of the insect intestine, but it may afford protection from desiccation and predation. These insects feed the substances in the xylem, the ascendant conducting system of both herbaceous and woody plants. Its known host range is vast, including more than 100 plant species. Excreta are therefore produced in high number (leafhopper rain) and become a white residue, which can damage the leaves of crops.

However, there is another explanation for the feeding behavior of the vector, leading to reasons for the disease. Xylematic nutrition should not be limited to the ascending lymph, ascending from the reserve organs, but it could interest also the phloematic conductive system. In fact, the active conducting tissues, xylematic and phloematic, are collateral and the two kinds of lymph flow very near. Furthermore, through xylematic feeding these insects can have access to the assumption of nitrogen in the form of amino acids coming from the symbiotic nitrogen fixation. This endosymbiotic input can compensate the nutritional imbalance by the assumption of large quantities of liquids and by the microorganisms' nitrogen fixation. The result is a physiologic-anatomic system of filtering and selection of the nutrients, and these insects can rely on a specialized structure of the digestive system, the filter chamber. However, the system recycles the ingested fluid and improves nutrient absorption, but it is not selective in the possible intrusion of bacteria, with physiopathologic effects. In fact, Cercopoideceous insects, including *X. fastidiosa*, are cited in the literature as vectors of phytopathogen microorganisms living in the xylem.

Feeding times and growing of the adults are thus orchestrated to coincide with the period of peak nutrient content in the host plant. That means an absolute need to study this coherence in the habitat, based on the knowledge of the preferred host plants and the behavior of the vector. Once again, we are looking to a complex system of interconnected microhabitats, including the acquisition of *X. fastidiosa* while feeding from an infected plant. As already reported, the bacterium can survive only in adapted special environments. It establishes itself perfectly inside the insect, attaching itself to the surfaces of the mouthparts of the vector, on the floor of the cibarium, the apodemal groove of the diaphragm, and in the walls of the precibarial area both above and below the precibarial valve. In this way, the bacterium can persist and be ready to transfer itself in the new host through the biting of the vector. Nymphs of *X. fastidiosa* lose their ability to transmit the disease after molting. Transmission to other plants takes place during the seasonal flights of the vectors, when they are in large numbers.

Considering the previous similar cases of insect-borne diseases concerning plants, there are two possible main future scenarios. The epidemic could stop by inner or outer mechanisms, or the infection could spread out of Salento. In the second case, some models predict a meridional expansion, with a rapid transit through Calabria and the invasion of Sicily and Sardinia, but later Corsica and any Mediterranean region could affected. At this stage, any neighboring territory is in danger. Nowadays, there are no reasons to consider the Xylella outbreak already finished, since there are several reports about the presence of the paradise outside the zone of restriction imposed by EU. The vectors are not able to fly for long distance, but they can easily overcome the 2 km line of separation in several ways. When I was in Salento at the beginning of the infection, the windows of our cars were full of these vectors, trying to achieve transportation. The jump of these insects, although remarkable in consideration of the dimension, could not be enough to rich distant places, but they are light and can be easily transported by the wind for long distances. If you are in Salento, you may be struck by the changes. The previous green has been totally substituted by the dirty yellow of a desert sandy land where only the enormous carcasses of destroyed trees are present, as silent testimonials to a catastrophic event. Meanwhile, the situation in Italy alarmed all countries where the olive trees are present. The main measure so far was a general plan of detection of the current presence of the bacterium. The detection involved also the other side of the Atlantic Ocean, even where probably all the story started with Pierce's disease of grapes and almond leaf scorch.

In California, the incidence of *X. fastidiosa* in field populations of other species of leafhoppers has been observed by Frietag and Frazier to be as high as 18.6% for most of the year. In Florida, in contrast, natural infectivity of the glassy-winged leafhopper is very low. As to the vector efficiency of the glassy-winged sharpshooter, Costa et al. (2000) found that, under ideal conditions, 83% of oleander plants each exposed to a single leafhopper carrying *X. fastidiosa* became infected with the bacterium.

The situation in the United States was monitored in 2014 and reported in P*lant Disease* by Krugner et al. (2014). In the USA, California is the sole relevant producer of olives, with ~17,800 ha planted and a production value estimated at US$130 million per year, although olive trees are also sporadically present in Florida. The study was promoted by increasing occurrences of dieback and leaf scorching, which were considered as symptoms in California olive trees, with *X. fastidiosa* (Xf) as the causal agent. In total, 198 samples of olive trees showing branch dieback and leaf scorch symptoms were collected. Analyses by polymerase chain reaction (PCR) were performed to determine the association of Xylella with the disease. In detail: "Laboratory tests detected Xf in only the ~17% of the samples, from which six strains of the bacterium were isolated. DNA analysis and laboratory tests using grapevines and almonds indicated that Xylella recovered from olive belong to a subgroup known to cause almond leaf scorch but not Pierce's disease." Bacterial cells from laboratory cultures were inoculated into healthy olive plants and monitored over 1 year for symptom development and presence of bacteria, which tended to be self-limiting. Tested olive plants did not show any symptoms of disease, and Xf infection tended to be self-limiting. Results indicate that although Xf is unlikely to be the causal agent of olive leaf scorch/branch dieback, the infected olive may serve as a reservoir for Xf and insect vectors, thereby contributing to the diffusion of Xf. These results were partially confirmed also for the analyzed affected plants in Apulia, where the presence of Xylella was not always confirmed.

However, the paper contains also another clear indication, even more important:

"Collectively, the data indicate that *X. fastidiosa* is unlikely to be the etiological agent of olive leaf scorch/branch dieback, but olive may contribute to the epidemiology of Xylellae-caused diseases in California. Olive may serve as an alternative, albeit suboptimal, host of *X. fastidiosa*. Olive also may be a refuge where sharpshooter vectors might escape intensive area-wide insecticide treatment of citrus, the primary control method used in California to limit glassy-winged sharpshooter populations and, indirectly, epidemics of Pierce's disease of grapevine."

This is in perfect accordance with our experiences in the laboratory and in the field. Let us imagine the life of the vector during the feeding period. Herbal plants are surely preferred for several reasons, considering that to feed on the olive tree it is necessary to penetrate the thick and resistant bark of the trunk, relying on a delicate and minuscule stylet.

Attempts to find solutions among the biological control give some results, consisting in a parasitic tiny wasp, *Gonatocerus triguttatus*, which could control the early spring generation of the sharpshooter. As is usual in these cases, the wasp from Texas and Northern Mexico parasites the eggs of the sharpshooter with its eggs. An alternative could be the utilization of entomopathogens, such as *Hirsutella* sp., a fungus that is known to affect sharpshooters. It is important to underline that these organisms are already present in the southeastern United States, although improving their spread could be very difficult.

There are interesting similarities but also some important differences in the comparison between the Californian and the Italian cases (Overall and Rebek, 2017). The six strains of *X. fastidiosa* isolated from olive trees in southern California pertained to the subspecies *multiplex*. The glassy-winged sharpshooter, *Homalodisca vitripennis* (Hemiptera, Cicadellidae), resulted among the vector involved by transmission assays. However, it must be underlined that it has been demonstrated that this insect could transmit strains of both subspecies *multiplex* and *fastidiosa* to olive trees at low efficiency. Using insect trapping data, other vectors were proposed, like *Draeculacephala minerva*. Therefore, so far there are not evidences about a general widespread of the OQDS outside Salento, but this is not a reliable alibi to diminish the attention.

If the soil is totally deprived of any herbal species because it is cleaned by a tractor, as in the usual tradition in olive field, the only way to survive for the vector, and the parasite inside, is the transferred to the woody plants. Cleaning of the soil is a usual treatment in order to facilitate collection of the olives and prevent the *Bactrocera olea* attack on olives, but even more effective again the survival of herbs could be a prolonged period of months without any rain and high temperatures, as registered in the last years in the south of Italy. A combination of these factors could be the reason for a change of feeding behavior by the vector. This hypothesis needs confirmation. Therefore, in the last 2 years in collaboration with the team at ENEA, as part of my research group, attention was dedicated to the determination of the preferred feeding species for *P. spumarius* in central Italy. The aims of the study were not only the determination of the preferred species, but also the period of feeding, the relation between insect and plants, the possible presence of alien species, and the environmental factors influencing the vector behavior. The last argument is crucial: if the neotropical climate will dominate in the Mediterranean basin, the adaptation of plants can be influenced. Now, most plants are autochthonous or were introduced a long time ago, but other types of adaptations are possible in future. For instance, the baobab presents leaves only for 3 months during the year, a period coincident with the rains, but in addition, the trunk under the bark possesses protected green tissues that are able to perform the chlorophyll synthetic process throughout the year.

The investigation of the host plants influencing the distribution of vector of *Xylella fastidiosa* and *Phylenus fastidiosa* was tracked on long-term field observation, monitoring the presence of nymphs on 144 different target herbaceous species by visual count of the insect feeding foams (Benelli et al., 2018). In addition to providing new insights about the feeding behavior of the vector, the study was performed to verify if the spatial distribution of the nymphs was influenced by olive tree proximity, in order to obtain information about a possible relation between the olive tree and *P. spumarius* (Dongiovanni et al., 2018, 2019; Bodino et al., 2019; Latini et al., 2019; Krugner et al., 2014). The result of the research was a predominant preference of the nymphs at IV and V larval stages on a very limited number of herbal species in comparison with the plethora of herbs present in the field, but with differences during the year. Each year in March, the fennel, *Foeniculum vulgare* (Apiaceae), and the white bedstraw, *Galium album* (Rubiaceae), were the most visited, whereas from April the preference changed according to the seasonal development, shifting mainly to the sticky-willie, *Galium aparine*, followed by the hawkweed oxtongue, *Picris hieracioides* (Asteraceae), *Silene latifolia* (Cariophillaceae), and the common sowthistle, *Sonchus oleraceus* (Asteraceae); however, infestation on fennel resulted relevant in the May. In general, although nymphs feed on the same herbaceous plants of eggs deposition, adults have polyphagous feeding behavior. Thanks to their improved flying and jumping abilities, they could feed also on other plants, like shrubs and woody trees. *P. spumarius* certainly displays a good adaptability and therefore data on the biology and ecology of its local populations are of pivotal importance in any control program. This consideration is necessary to continue this narration, and to join the data from U.S. researches about Xylella occurrence with the Italian observations on vector feeding behavior.

We have seen that fennel is a key herbal species for diffusion of Xylella vector. It is evident that when we are talking of sweet fennel, we are referring to the wild plants very different in shape and constituents from those that we use for culinary purposes. The wild plants are present in the fields in summer, often as a highly invasive weed. In fact, from the yellow-green umbrella flowers, typical of the Apiaceae (Umbelliferae) family, at the top of the plant from late winter to summer, a single plant can produce thousands of seeds during its first growing season and hundreds of thousands of seeds in its second year of growth, multiplying its presence easily. Therefore, thanks to these characteristics and the long fruiting period, it is a strong candidate to become invasive and dominant, disturbing plant communities and habitats. As confirmation, *F. vulgare* is included in the CABI *Invasive Species Compendium*.

Fennel originated from South Europe, in particular with an areal distribution similar to that of the olive tree, but it was introduced in several temperate regions around in the world, including North America, and it is now known as wild anise in California. Owing to its typical strong smell, it is often mislabeled as anise in American supermarkets. In the USA, it can be found growing in San Francisco and on the Pacific coast, where it is considered a weed. After its introduction and naturalization in the 1880s in California, fennel escaped from cultivation, becoming a seed contaminant and an invasive plant. An alien invasive plant competes with other native plants for resources, making it harder for other plants to obtain sunlight, water, and nutrients, because the alien plant tends to take over.

In 2014, *F. vulgare* was included within the EU list of plant genera and species, as regards the need of measures to prevent the spread of the fastidious bacterium, and the relation between the bacterium and wild sweet fennel has been confirmed in other studies. Therefore, *F. vulgare* must be considered a European native emerging alien invasive species, potentially dangerous, also considering that so far the adopted measures to limit its spread have been unsuccessful. In other words, considering the proven existing relationship between the plant and the vector, confirmed by studies in 2018 (Morente et al. (2018) in Spain and Dongiovanni et al. (2018, 2019) in Apulia), the wide spread of wild sweet fennel colonizing new territories could contribute to the spread of diseases carried by the insect.

Finally, two additional considerations were included in the work of ENEA researchers. The above data outline the relevance of specific wild herbaceous plants for survival and growth of the most vulnerable stages of the vector. In olive cultivations of south Italy, the consolidate habitude of clean the soil from weeds in olive fields may be favorable to the vector, as already suggested. This treatment can favor the re-growing of wild fennel with the laying of eggs in autumn and cause ailments in nymphs in the hatching period.

In the last decades, many olive orchards in Apulia were abandoned, and the long tradition of careful cultivation was interrupted, favoring the weeding of uncultivated green areas. This is an additional potential factor of the OQSD outbreak, favoring the presence of invasive herbal species and alien vectors. The establishment of smart treatment to limit the plants,

Fig. 5.25 An olive tree significantly damaged by Xylella. The abundant production of shoots in the basal part is not useful to restart the tree's metabolism.

attracting the vector for feeding or egg laying, could make it possible to limit the utilization of insecticide (Fig. 5.25).

The future of olive trees

The Xylella alert and related events demonstrate the difficulties of governments and international agencies in adapting their strategies in the case of novel and unexpected alerts. It recalls in some ways the beginning of the movie *Godzilla*. When the Leviathan monster emerges from the sea and attacks Tokyo, the authorities spend a lot of time in interminable meetings, looking for a paragraph in the protocols where they might find written what to do in such cases. Godzilla is a special case and there nothing about an emergence like this in the protocols. Meanwhile, heedless of the bureaucratic impasse Godzilla continues its systematic destroy of the town. Against

Fig. 5.26 This is the end of an olive tree, as has happened in Apulia so far.

the unexpected attack of Xylella, the only adopted and strictly supported measures were ordinary ones foreseen in any kind of infection emergency, consisting of the removal of the infected organisms and acting to limit the zone of contamination.

So far, the only real performed action was the application of the EU protocol consisting of the eradication of any olive tree and creating a defensive line of 2 km in extension in the northern part of the Salento Peninsula, where any plant must be eradicated, in order to isolate the disease. This measure should stop the diffusion of *X. fastidiosa*. The blocking of plant imports from America is also in action. In addition, in this case, total control is quite impossible. It is very difficult that the EU's protective approach will have significant effects. The vectors are not good flyers; they usually move by jumping, but they can be efficiently transported by the wind and occasionally human or animal transportation, traveling kilometers in a single day.

Several aspects of the Xylella affair are waiting appropriate responses. We have seen the particular phytogeographic situation of the Salento Peninsula. It is possible that its partial isolation facilitated the diffusion of OQDS. Another agent could be assigned to the olive monoculture. Furthermore, as in many other cases, the environmental changes in action can be considered decisive for invasion or the increase of parasite virulence. Another influence deserving to be considered is the abandonment of agricultural care in many fields and the consequent degradation of the soil. Therefore, the absence of traditional treatment of the soil and the trees, due to urbanization of the population, could have interfered. We are probably facing a cooccurrence of all these factors and others as yet unknown. There are some unclear aspects of OQDS that were and are objects of speculation, such as the starting episode of the disease, and why olive trees were the selected target. In summary, OQDS must be considered a further edition of the PD pathology or a novel disease, which means an evolution or something never experimented. This is very important to understand the future of olive species and the possible effective measures.

In conclusion, there are two main hypotheses about future scenarios:

(a) a natural stress-induced dieback: this is consistent with widespread groves of various ages all suffering to different degrees and slowly declining rather than a virulent point infection that can be seen to spread. In other words, the disease is due to a "normal" increasing of virulence coupled with the "stress effect," derived from climate change and agricultural loss, that will affect mainly old trees, causing a turnover in favor of the new stronger generations;

(b) a modified, more virulent pathogen appeared, and plants defenses will not be able to face the new challenge, with devastating consequences.

The bacterium cannot be controlled by the use of chemical antibiotics, either because they are banned in agriculture in EU or because they are very costly and complicated. The insect vector could be controlled by appropriate insecticide, considering the environmental impact. Insecticides usually select as their target adults, but the larval stage is the best point at which to act on the insects, before they are able to move and fly away. In any case, it is fundamental to know exactly the vector's activities and plans in terms of where it feeds and reproduces.

Therefore, to obtain any real result, we must learn nature's lesson. Olive tree disease shows three main actors: the bacterium, the vector, and the plant (and probably a symbiotic fungus). Three fungal species were associated with the symptoms: *Phaeoacremonium aleophilum*, *Neofusicoccum* *parvum*, and *Pleurostomophora richardsiae*, although their effective roles are under discussion.

The associated organisms work together in the insect-borne disease, acting like a "superorganism." It is a very complex system, but in some ways also very efficient. The only way to face the *X. fastidiosa* challenge is through integrated pest management. It is necessary to operate considering the several aspects involved together: a treatment of soil to sustain the plant and its capacity to react to the disease; an insecticidal agent to control selectively the insect; and a natural, low-cost, and eco-friendly antibiotic treatment of new generation. Some may argue that this strategy is not so different from that suggested in the case of insect-borne diseases affecting mankind.

A preliminary key step is the reply to the question: how did *X. fastidiosa* become so dangerous in the last 2 years? We have known about the presence of bacterium in Italy for at least 30 years, and previously it was considered just one of the several diseases involving olive oil. Something happened in recent years changing completely the equilibrium between the microorganism and the host. There are several hypotheses about the causes of the change and a consequent relevant debate.

The second hypothesis is that a change in the *X. fastidiosa* genome occurred, giving rise to more aggressive and dangerous strains not present in the past (Steinbiss et al., 2016). In this case, there are two possibilities: the change is derived from some experiment or a biological cause. In the first hypothesis, a genetic laboratory generated a mutant population, which later escaped its creators. In the second hypothesis, due to climate change, some virulent strains from hotter countries were able to survive and proliferate against local populations. A conjecture is based on the possibility that the parasite was introduced through the import of infected plants. In fact, the hypothesis of the import of infected ornamental plants, like oleander from Costa Rica, has been seriously considered. However, it is complicated to imagine that all the imports of the infected plants were concentrated in Gallipoli and that they contained a special highly aggressive strain of the bacterium never previously reported. In any case, the cooccurrence of several improving basic factors must be considered, including climate changes.

The climate hypothesis

There is a public debate about the potential impact of climate change, in particular global warming, on human health and the future of the planet. However, there is often confusion about the usual terms, like climate (from the Greek *klima*, meaning inclination). Besides the influences due to the human activity, other factors can be considered as causes of the climatic variation: natural factors like the sun's radiant energy; alteration of the hearth's axis causing variation of its orbit. The effects of these factors on the climatic elements of the environment, such as temperature, humidity, wind, and rainfall, can cause significant local changes, but climate tendencies must be considered in averages for decades, centuries, and even millennia. The problem is that often the climate is confused with the weather, which actually refers to short-term climatic situations. This is the consequence of the weather's impact on our ordinary life conditions. In other words, people focus on updated forecasts, like the temperature in the next days or the possibility of rain. These are local and transitory situations, but more consideration should be dedicated to long-term aspects. This confusion is increased by continuous catastrophic warnings online and so on about extreme consequences in our lives every summer or winter, just to encourage us to click. It is pointless to insist that any forecast on the next week is not trustworthy, whereas the tendency of the global climate can be considered more reliable. In any case, meanwhile we discuss if the range of years is too short to decide if the warning current trend is a minor fluctuation or a first-order variation, we can observe the consequences of climate change on our habitat, including on insect-borne diseases. The debate on effects on climate changes is ongoing, increasing on one side the interest of ordinary people, but also the consciousness that the measures so far claimed are not adequate to the situation.

There is general concern about the recent increases of the global temperature and of carbon dioxide in the atmosphere. Data evidencing this reality and the relative impacts of the climate changes can be easily found in the reports of international agencies. Thus, United Nations (UN) at its annual 2019 convention reported fundamentally a global increase of the temperature of *c.* 1.5°C in comparison with pre-industrial times and an exponential increase of the percentage of CO_2 in the atmosphere. These increases are still considered acceptable, but the preoccupation concerns the effects of a confirmation of the tendency in the next decades. In 1901–2012, the increase was 0.89°C and a further increase of 0.3–0.7°C is expected in the period 2016–2035. A total increase of *c.* 3.7°C is expected. The population directly involved in the phenomenon is evaluated: 178 million for +1.5°C, 200 million for +2°C and 277 million for +3°C. We can assume that all these people will have only two possible futures: succumb or migrate, unless something changes the scenario.

The increases of temperature and CO_2, as well as the different distribution of precipitations, are considered the results of abiotic and biotic factors. About abiotic factors, like a change of magnetic field of the planet, we can do nothing and therefore attention is concentrated on biotic effects, although these are difficult to establish. Among the human influences, industrial and domestic pollution have been considered as mainly responsible for climatic change, and it is believed that a decrease of industrial activity could influence significantly the increasing tendencies. Governments are requested to act on pollution caused by urbanization, i.e., cars and houses heating, usually derived by changes of fuel. However, other effects are in action and these can be decisive as well. Larger populations need more food and cultivable lands are under increasing pressure. Intensive agriculture and the utilization of soil are responsible for at least 23% of biotic contribution to the greenhouse effect. Soil is continuously impoverished by intensive agricultural systems and the increase of CO_2 affects the nutritional value of food (−5.9%–12.7% in proteins, −3.7%–6.5% in zinc, −5.2%–7.5% in iron). Insect-borne diseases are mainly considered in terms of their effects on human health, but they are key factors on the production of food and its quality. Among the factors, influencing the evolution of food, the insurgence of organism genetically modified and the concentration of production and distribution under the control of multinational agencies. The consequences of these factors were the decrease of biodiversity and of number of varieties, as well the loss of local production, which are the result of a long and accurate selection.

The problem is the speed and the entity, not the nature of the phenomenon of climate change. Turnover of hot and glacial periods is a normal trend for our planet, mainly due to the quantity of CO_2 in the atmosphere. We know the occurrence of several marked changes in climatic conditions in Europe during the last 1000 years, mainly attested by the alternant records of temperatures. These changes deeply influenced human activities, like in the cold medieval period, when it was difficult to survive because of the low temperatures, which affected agriculture and crop production. Later, the re-increase of the temperatures allowed an astonishing renaissance of human activities, in sciences, arts, architecture, and other expressions of mankind's intellectual performances. In the 20th century, two main periods of warming have occurred in Europe. A second period of warming started in 1976–2000, recorded as the warmest one, and it is still in action. The evidence was an increase of approximately 1.2°C over the past 100 years, which is twice the average global rate. We are now in a warming period, and the trend is probably accelerated by the emissions due to human activities. Warning consequences are higher nighttime temperature, with limited differences between day and night, and fewer frost days in winter, associated with milder temperatures throughout the winter period, longer dry periods, and peaks of temperature. However, if we consider only the data referred to the tendencies of global planet changes, we can extrapolate a limited, even misleading, impression. In particular, considering Europe, we must focus on opposite effects: temperature increases were most marked in both Central Europe (Italy, Corsica, and the Balearic Islands) and Eastern Europe (western Bulgaria, northern Greece, Albania, Macedonia, Bosnia, Montenegro, and Croatia). In contrast, central Iberia and the region around the border between Morocco and Algeria have cooled. Simplifying the tendency obtained from models, Europe is warming and North Africa is cooling. This has resulted in changes in precipitation dividing Europe into two parts: the number of wet days have increased in North Europe and decreased in South Europe, increasing the existing tendency to desertification of several regions. North Europe, including the UK, northern Iberia, and Scandinavia, is becoming wetter, whereas southern Iberia, France, Germany, and Italy are becoming drier. The main problem in all this is people's attitudes, virtually all oriented to the defensive. However, we must consider seriously the necessity of adapting our strategies to climate change, and even consider the possibilities of obtain working places and inputs for the economy. I suggest abolishing the word "conservation," so beloved by my ecologist colleagues, usually the preview of a useless and unsustainable position of a rearguard. Vector-borne pathogens are particularly sensitive to climate, a fact that has led to widespread and continued speculations that anthropogenic climate change will increase the incidence and intensity of their transmission. Other climatic abiotic and biotic factors can also affect disease distribution. Diffusion can be very rapid and effective. Adult insects are usually not strong fliers, but they can be passively dispersed by the wind, possibly up to several kilometers in a single night, especially over the sea. Thus, natural barriers cannot be considered an efficient control of the diffusion. Otherwise, these insects can travel utilizing ancient transportations, like other animals (street ruminants), or new unexpected ones, e.g., inside old tires as happened for *A. albopictus*.

There is an urgent need for ecologically sound, equitable, and ethical pest management, based on control agents that are pest-specific, nontoxic to humans and other biota, biodegradable, less prone to pest resistance and resurgence, and relatively less expensive. The last aspect is fundamental for large-scale use in emerging countries.

The general feeling is that we are facing something exceptional and never before seen, but history tells us another tale.

It happened before, and it will happen again

History books are full of facts, but very few contain information about reasons for certain events, in particular when they are not referring to physical-chemical implications. Everything seems to be the consequence of human decision or of the arising of important personages influencing the destiny of communities. Nevertheless, we can reverse the paradigm and consider human activities as the result of the effects of natural forces.

Many history books begin with the story of two empires@ one in Egypt and the other in Mesopotamia, the land between the Tigris and Euphrates rivers. The Akkadic Empire began in the cradle of our civilization, wherein an alphanumeric system and geometry were already known, as well as advanced social organizations. In 4500 BCE the great towns of Akkad and Babylonia lost their independence to King Sargon, who was able to conquer other large territories. This led to the foundation of the first "universal" empire, meaning a single political-territorial entity, united despite the great geographic, ethnic, and cultural diversities. The northern part of the empire was relatively rich, profiting from fertile and productive fields, whereas the southern regions were semi-arid. Starting from the cornerstone in Mesopotamia, the empire extended to what are now Syria, Turkey, Iran, Iraq, and Saudi Arabia, conquering most of the civilized lands on the planet. However, after 150 years the Akkadic Empire collapsed (Weiss et al., 1993; Weiss, 2017). The reason for its disintegration is usually assigned to undetermined invaders from the north, pressing people to the south. However, something in this seems difficult to accept. The empire was already well-established and the populations in the northern regions should have had low interest to change in favor of the southern territories. Even in the case of an invasion from other populations, considering the empire's wealth, assimilation by the invaders appears more probable. Scientists started to speculate on the case, searching for evidence of the real sequence of events. This time, the research was focused on traces of very old time. It was a difficult and specialized search. Since the old reports were contradictory, researchers looked for indisputable traces, like those trapped in the rocks (Zanchetta et al., 2016; Kornei, 2019), consisting in evidences able to resist during 4000 years to the atmospheric aggressions and environmental changes, including the human activities. Although we have encountered so far towns, fertile fields, and arid deserts, the scene was taken by a deep cavern, wherein the scientific team, whose leader was the paleoclimate expert Stacey Carolin (Carolin et al., 2019), University of Oxford, found the trace they wanted (published in PNAS in 2019). Inside the cavern of Gol-e-Zard (*c.*5000 m distant from Teheran and protected by a great mountain from the northern winds), the team was able to obtain a sort of archive of the climatic changes in that area. They obtained information, measuring the quantities of magnesium present in the stratification of the stalagmites produced by the deposition of minerals coming from the water percolating from the upper soils. This is a reliable method to measure the quantity of rain and powder present on the surface of an exposed area over a determined period, but these data must be cross-referenced with the chronologic system of dating, based on uranium/thorium concentrations. In such a way, the team obtained precise information about the quantity of powder and sands present at a determined time in the zone, as well as the quantity of rain. The results evidenced the occurrence of two long periods of extreme drought; the first one started in 4510 BCE and lasted for 110 years, and the second started in 4260 BCE and lasted for 290 years. These data were confirmed by other independent researches, such as those on corals. The idea is that powder and sand were transported by the wind to be deposited in such quantities as to make any cultivation impossible, and the fertile fields of the north thus became unproductive. In such a way, the previous equilibria between north and south were completely thrown out of balance, causing a great migration of people in search of food. Once the scientific determination was obtained, it was possible to compare this with historical information. The beginning of the second period coincides with the start of the collapse of the empire. In a fragment of a document of that period, named "The Damnation of Akkad," we can read: "the great cultivable fields were not able to produce wheat any more, the flooded fields did not produce fish, the orchards did not produce syrup o wine, no rain from the dense clouds." This may have been the first human mass migration registered in history as caused by climate changes. The rest of the story is always the same. The exodus from north to south generated conflicts with the local populations, and the consequence was the construction of a wall 180 km long between the Tigris and Euphrates, which was named the "Wall of Amorrei."

The Trump Wall is planned to run for *c.*3000 km, coast to coast. Already a third of the boundary is divided by various kinds of barrier, for pedestrians and/or vehicles. The wall is going to cross several types of habitats, such as mountains, rivers, deserts, and plains. Now, we can try to leave aside any political considerations (fortunately they are beyond the aims of this book) and the environmental effects (there is always an ecological impact) involving more than 200 plants and animal species (according to the online 2016 Information for Planning and Conservation) and the production of 7.4 million tonnes realized in the atmosphere, the consequences of genetic breeding, and many other arguments. We can consider everything as a necessary collateral effect, and let us evaluate only whether the wall will be able to act as an effective antimigration barrier, and thus achieve its objective. In the light of what is going on in other countries, stopping terrestrial human migration is not easy, but is arguably largely possible. However, there are then the coasts and the seas. In particular, California's coast is highly populated and extended, with plenty of transportations possible through water. A confirmation of this trend comes from the situation in Europe, with a continuous migratory flux from Africa. Nobody, so far, apart the official declaration of hostility against migrants, was able to stop it and avoid the loss of lives during the desperate trip. How many boats or other kind of ships full of migrants were prevented from reaching Italian harbours? As far as we know, the number is zero. There are many ethical and juridical considerations, but once again the physical concern is the main one. From the point of view of insect-borne diseases, the Trump Wall can have few effects, since insects can fly and eggs can easily be transported by the wind and by any type of vehicles—unless the wall will be able to stop all molecules.

The role of secondary metabolites

Four olive cultivars, Cellina di Nardò, Ogliarola di Lecce, Frantoio, and Leccino, resulted positive in response to the Xylella infection, accounting for most cultivars present in Salento. However, the response to the disease was not equal, based on the qPCR assays. Cvs. Cellina di Nardò and Ogliarola di Lecce showed higher disease resistance than Frantoio and Leccino. The HPLC-ESI-TOF-MS analysis showed a correlation with modification of phenolic content, focusing on two key metabolites: hydroxytyrosol and quinic acid. As evidenced in a parallel study on grape cultivars, these substances act as a reservoir in the pathway production of a quantity of phenolic compounds, whose importance in antioxidative and defensive mechanisms is well-known. In particular, constitutive levels of hydroxytyrosol glucoside turned out to be strongly reduced in infected plants (more than 94%), although the effects were greater in cvs. Frantoia and Leccino. Regarding quinic acid, the situation was different: levels did not indicate a significant change in cultivars, but in the infected aforementioned cultivars the contents of quinic acid increased four- and five-fold. The results refer to the leaves, which are most affected by the disease. It is conceivable that these intermediate metabolites, in the case of an attack, are converted into other derivatives. Hydroxytyrosol glucoside is a precursor of oleuropein, which can be considered the most important secondary metabolite in olive production for its antiROS properties. Quinic acid is fundamental in the production of caffeoylquinic compounds, also considered important for the same reasons. These studies suggest a significant response of the infected plants, based mainly on the secondary metabolism, as expected, and could explain the different sensitivities of species and varieties. This molecular response by the plant could be improved and offer the key to the tree's capacity to react to the disease.

Another consideration concerns information about the possible manifestation of OQDS in other parts of the planet, as well as differences in evidence of the disease. California is the sole producer of olives in the USA with ~17,800 ha planted and a production value estimated at US$130 million per year (Overall and Rebek, 2017). Increasing occurrences of dieback and leaf scorching symptoms in California olive trees suggested the possible occurrence of OQDS in California (Purcell and Saunders, 1999). A total 198 samples of olive trees showing branch dieback and leaf scorch symptoms were collected and investigated, but in only 33 samples (~17%) was it possible to detect the presence of the bacterium. These data were confirmed by DNA analysis and laboratory tests on grapevines and almonds, showing that the Xylella strain recovered from olives can be assigned to a subgroup known to cause almond leaf scorch but not Pierce's disease. Furthermore, bacterial cells obtained from laboratory cultures were inoculated into healthy olive plants. After 1 year, the analyses of tested olive trees did not show any symptom of disease development and the infection tended to be self-limiting. All the experiments indicated that Xylella is unlikely to be the causal agent of olive leaf scorch/branch dieback, which must thus be attributed to other agents or causes. Nonetheless, it is conceivable that infected olive trees may serve as a reservoir for the bacterium and insect vectors, contributing to the epidemiology of diseases caused by Xylella.

The research on California olive trees also demonstrated by vector transmission assays and insect trapping that the glassy-winged sharpshooter, *Homalodisca vitripennis* (Hemiptera: Cicadellidae), and green sharpshooter, *Draeculacephala minerva*, were active in olive orchards, contributing to transmission of strains of both subspecies *multiplex* and *fastidiosa* to olives, though at low efficiency. Another approach was inspired by the consideration that olive trees may serve as an alternative (albeit suboptimal) host of *X. fastidiosa* in the case of absence or low availability of the preferred hosts.

Conclusion

In 2013, I was among a team of experts participating in a restricted first meeting to evaluate the Xylella problem. My opinions, not accepted by the other participants, were as follows: (a) the situation must be interpreted as a severe epidemic; (b) the attack cannot be solved only by the ordinary methods, but needs an extraordinary effort to produce new solutions; and (c) considering the relevance of the situation, a program of information and general mobilization of farmers and the population should immediately start. Regarding the first consideration, everyone can see directly what happened by looking at the photos and reports (Figs. 5.25 and 5.26). As for the second, we will see in the near future, but so far the signs are not positive. About the third one, we lost 2 years to silence and when the alert exploded, the result was that the population was against eradication, with the usual pathetic solution of reimbursing farmers for the damage. Nobody will reimburse all of mankind for the environmental damage. Meanwhile, smart merchants, like vultures, are ready to profit of the disaster, selling cultivars of olive trees, presented as untachable by the Xylella.

The moral of the Xylella affair is clear. The good news is that we have powerful methods to detect the presence and diffusion of the pathogen's strains. The bad news is that the chances of preventing or predicting an invasion are still practically zero, as evident in the current situation (Fig. 5.27). Therefore, we are still able to act when there are signs of emergence. The terrible news is that pathogens are very fast indeed compared to our capacity to respond. Most insect-borne diseases are decreasing, but this is probably dependent on mankind's health, and the other fronts are in a different situation. The research front is moving in accordance with requirements and producing valuable proposals, but it needs financial support and, more importantly, the chance to realize concrete results.

Fig. 5.27 The current look of several parts of Apulia after the Xylella attack.

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