Thrips–tospovirus interactions: Biological and molecular implications

T. N. Ananthakrishnan[1,*] and R. S. Annadurai[2]

[1]Flat No. 6, 'Dwaraka' 22 (42), Kamdar Nagar, Nungambakkam, Chennai 600 034, India
[2]Vittal Mallya Scientific Research Foundation, P.B. No. 406, K.R. Road, Bangalore 560 004, India

The occurrence of thrips vectors in considerable numbers enables their functioning in a dual role as vectors and as direct crop pests. The resistance of thrips to pesticides has enabled quick transmission of viruses, the transient nature of their populations being essentially responsible for the infection. The feeding behaviour of thrips contributes in a large measure towards their ability to act as vectors, enabling leaf-to-leaf transmission of the tospoviruses. The specific association of the tospoviruses and thrips vectors, particularly relating to the molecular profiles, needs increasing scrutiny to come to proper conclusions. A better understanding of the nature of virus multiplication and the pathways leading to their entry into the salivary glands and the ability of the second instar larvae to inoculate plants need further inputs. The intraspecific diversity of thrips vectors as a result of population studies from various parts of the country, would further enable a better understanding of the ability of each species to transfer the virus, besides better appreciation of the chemical ecology of thrips–host-plant interaction, not to mention the relevance of serodiagnosis in detecting disease or health.

Keywords: Crop pests, host-plant, thrips, tospovirus, vectors.

THE adaptive diversity of thrips has enabled successful exploitation of diverse niches resulting in their establishment in a variety of plants. The increasing evidence of thrips infestation in various cropping systems, their ability to migrate from weed reservoirs to crops and vice versa, their intercrop movement and related adaptive strategies are basic aspects of thrips bionomics. Their abundance and patterns of distribution on various host-plants involving both crop and weeds are important factors in their population dynamics. Successful interactions of thrips with their host-plants appear to depend on a complex set of environmental, visual, tactile and chemical factors that appreciably influence their behaviour and physiology. Monophagy, oligophagy and polyphagy appear to have ecological implications, so that the condition of the host-plant is a major factor for successful exploitation of various host-plants. In this process, thrips develop structural, physiological and behavioural diversities enabling them to behave differently in diverse habitats at different periods of development and under varying environmental conditions[1]. Synchronization of the flowering periodicities with the emergence of new generations, appears to favour thrips populations, which extensively feed on tender developing parts inflicting severe damage to tissues leading to bleaching, necrosis and leaf and bud-shedding. Morphological complexity of the host-plant offers greater diversity of potential niches, the number and location of which tend to influence successful host-plant switching. Suitability of the host-plant therefore depends on whether the colonizing species are utilizing the plant for foraging and oviposition, and whether the larvae are capable of completing their normal growth and development in the same host-plant which the adult female chooses for egg-laying[1].

Therefore, the stage of development of the host-plant and time of attack, source of information and build-up of thrips populations, host-plant strategies in the regulation of thrips populations and nature of host responses are essential criteria for thrips–host-plant interactions[2].

Feeding dynamics

Following surface exploitation, internal exploration results in stylet penetration due to which plant fluids enter the precibarium and subsequently into the cibarium. Thrips must take up plant fluids into the precibarium and cibarium to discriminate between host-plant and feeding sites. With the downward thrust of the head, the mouth cone compresses against the substrate surface and mandibular and maxillary stylets pierce the substrate. The maxillary stylets form a groove through which the cell contents are sucked up. The impact of feeding behaviour, whether shallow or deeper probes, is an important factor in virus transmission. When thrips feed with pushes of short duration they empty their contents with small groups of cells, while probes of longer duration with a longer period of injection tend to be destructive to leaves so that viruses are transmitted through brief periods of shallow probes[3,4].

Vector–virus relationships

The ability of thrips to act as vectors of plant diseases has opened up a host of problems relating to thrips–host plant

*For correspondence. (e-mail: ananththrips@yahoo.com)

interactions. Extensive host-plant ranges are very typical for thrips survival as vectors, with the ability of both thrips and viruses to survive in diverse climates. The close association of many pathogens with insect vectors provides a versatile means of spread, achieving an efficient distribution of inocula. Such a close association has given the three components, viz. vector, virus and host-plant the status of an 'inseparable ecological trinity'[5]. The *modus operandi* of exploitation is also achieved by their polymorphism with the production of diverse adult forms involving apterous, macropterous forms besides colour differences. Species-specific responses necessitate in-depth analysis through behaviour models. In this context one needs to recognize morphological, physiological and genetic polymorphisms that contribute to the ever-widening trophic diversity, from oligophagy to polyphagy. The rate of adaptative changes influences colonization, exploitation of the host and subsequent dispersal patterns. Several intrinsic factors such as metabolic adaptability of thrips as well as extrinsic factors operate in relation to host suitability[1].

Tospoviruses (from the name Tomato Spotted Wilt Virus, TSWV) belonging to the family Bunyaviridae, are the only viruses infecting plants. Bunyaviridae viruses are recognized by their single-stranded RNA in three genome fragments, all held together in a membrane-like envelope[6]. The three RNA strands in the virus genome are called large (L), medium (M) and small (S), due to their size, i.e. 8.9, 5 and 2.9 kb respectively[7]. The negative sense L-RNA codes for the RNA-dependent RNA-polymerase (RdRp) are necessary for further replication[8]. The ambisense M-RNA codes for G2–G1 polyprotein are used in the envelope and a NSm protein involved in cell-to-cell movement[9]. The ambisense S-RNA codes for a nucleocapsid protein (N) and a NSs protein with unknown function[10] (Figure 1).

Many species of thrips are presently known as Tospovirus vectors transmitted from plant to plant by species of thrips mostly belonging to the genera *Thrips*, *Scirtothrips* and *Frankliniella* which are identifiable by their colour, body setae, wing setae and presence or absence of a comb on the VIII abdominal segments.

The relationship between vector/virus being specific, only the larval stages acquire the virus, though both larvae and adults have the ability to transmit the virus. The host range of the virus is considerable, mostly infesting major crops like tomato, potato, cucumber and peanut, to mention a few. Many tospoviruses infest weeds, so that weed–host-plant–vector interactions become important. TSWV belongs to the family Bunyaviridae and on the basis of serological studies and amino acid sequence of viral proteins fourteen species of TSWV are recognized. Some of them are watermelon bud necrosis virus, tomato chlorotic spot virus, peanut chlorotic virus, melon yellow spot viruses, groundnut ring spot virus and groundnut bud necrosis virus[11].

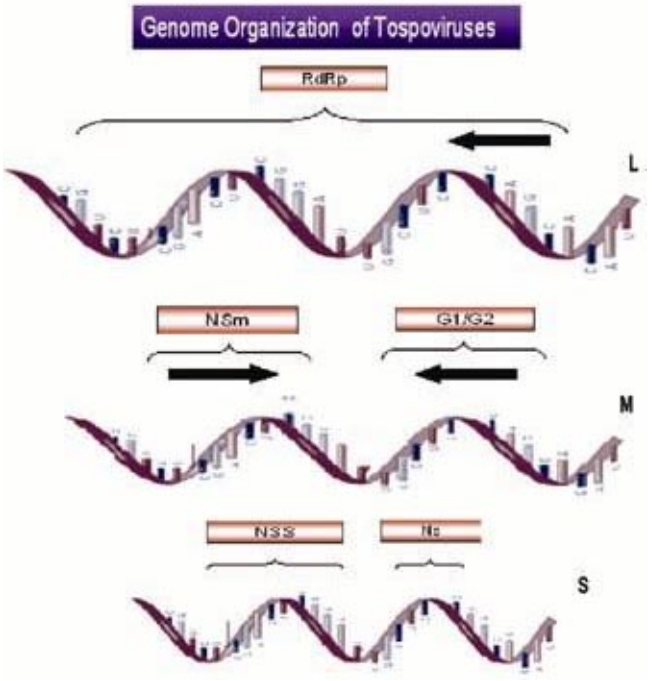

Figure 1. Schematic representation of the viral (L – large, M – middle and S – small) genome. Arrow represents ambisense genome organization for nonstructural (NS) proteins of M and S located in the 5′ region and structural proteins (N and G1/G2) in the 3′ region. (Source: APS net feature, plant pathology on-line.)

The major Indian thrips vectors are *Scirtothrips dorsalis*, *Frankliniella schultzei*, *Thrips tabaci* and *T. palmi*. Tospoviruses infect insect cells by binding to a host cell receptor through the mediation of a viral surface glycoprotein. Viral replication in the context of plant infection shows that viral replication takes place in the vector also. Vector competence is adjudged by the rate of virus multiplication in the mid-gut, and extent of migration from mid-gut to visceral muscle cells and salivary glands is crucial in the determination of vector competence. One of the main factors affecting vector competence and efficiency relates to the amount of TSWV in the adult thrips and the rate of replication in the tissues of thrips, notably the midgut and salivary glands. Sometimes barriers exist preventing failure of thrips populations to transmit the virus. This is exemplified in the failure of thelytokous *T. tabaci* populations to transmit the virus[12].

There is a sort of co-evolution between tospoviruses and vectors, and the striking morphological diversity of the vectors is suggestive of genetically variable populations. A good example is *T. palmi*, which is known today to transmit several newly emergent tospoviruses in cucurbits. Polyphagous thrips like *S. dorsalis*, *T. tabaci*, *F. schultzei* and *T. palmi* increase dispersal of viruses and with time and expansion of host ranges, chances of persistent viral transmission are enhanced. In view of the increasing incidence in thrips–vector interactions, the need for an integrated disease-management approach becomes

relevant, especially in relation to the molecular aspects enabling viral acquisition, movement within the vector and transmission.

Pathways of infection

As to the pathway by which the salivary glands become infected, the viruses tend to migrate from the midgut through the haemocoel to the salivary glands. Of particular relevance is the discovery of a second pathway involving translocation of the virus via a thin ligament connecting the midgut and salivary glands[12]. This lends support to the view that salivary glands become infected as a result of migration involving the ligaments, an aspect confirmed in *T. tabaci*. Primary infection occurs in the midgut epithelium when larvae have access to the virus early in development, the virus accumulating at different rates in the midgut epithelium.

To enable transmission, the virus has to reach the salivary glands before pupation, the adults directly acquiring the virus, and not transmitting the same. The only factor determining vector competence therefore depends on the successful replication of TSWV in the host. However, the rate of infection of the midgut muscle cells tends to differ in the different species of thrips. All the same it is now confirmed that infection in the ligaments preceded that in the salivary glands and that salivary gland infection is always preceded or accompanied by ligament infection. The ability, inability or partial ability to transmit the virus depends on the degree to which salivary glands become infected before pupation[12,13].

Vector capability

There is a sort of co-evolution between tospoviruses and vectors, and the striking morphological diversity is suggestive of functionally diverse populations. A good example is *T. palmi*, which is now known to transmit several newly emergent tospoviruses in cucurbits. Polyphagous species like *S. dorsalis*, *T. tabaci*, *F. schultzei* and *T. palmi* increase dispersal of viruses, and with time and expansion of host range, chances of persistent viral transmission are enhanced. In view of the complexities involved in the thrips–vector interaction, the need for an integrated approach becomes relevant, especially in relation to the involvement of molecular aspects including viral acquisition, movement within vector and transmission.

Regarding vector capabilities, it has been known that transmission capability of *T. tabaci* depends on isolation of TSWV as well as strains of the thrips. Vector capability depends on the types of *T. tabaci* in the population, and at present two types are known: *Thrips tabaci tabaci* on tobacco as well as potato and *T. tabaci communis* living on diverse plant species, mainly onion[11]. Variability in vector capability depends on the ability of *T. tabaci* populations to breed on weeds, and propagate by arrhenotoky, when transmission occurs; those which propagate by parthenogenesis do not have vector potential[18]. In *T. tabaci* populations only males are able to transmit the virus. At present, our understanding of the population dynamics of vectors such as *T. tabaci*, *T. palmi*, *S. dorsalis* and *F. schultzei* is meagre and analysis of populations and consequent sex-ratio becomes obligatory before any attempt is made to discuss vectorial capacity. *T. palmi* is known as the primary vector of Groundnut Bud Necrosis Virus (GBNV) in India, with *F. schultzei* transmitting the virus at a low rate, while *S. dorsalis* is known to be a non-vector[14]. Towards this end, the need for such studies from different geographical areas in the country, both from crops and weeds, becomes necessary. This calls for an integrated effort and adequate funding to be able to assess the functional diversity of vector species. The polyphagous potential of thrips and transfer from weeds and a host of vegetable crops within the same area tend to augment fast virus spread. Needless to indicate that intensive studies on larval identification as well as experimental induction of virus become obligatory. Therefore, analysis of the complexity involved in TSWV in transmission is important for disease management. Viral selectivity of larval thrips, borrowing time for multiplication through the pupal stage and subsequent transfer to a polyphagous adult seem to be a highly sophisticated natural programming. The events are intricately linked to one another, but the programming seems precise. Thrips being inconspicuous polyphagous pests as well as vectors with a high reproductive potential, an efficient transmission to the crop plants is guaranteed.

Biochemical profiles of host-plants and vector responses

Physiological and biochemical adaptations of thrips are directly correlated with the complexities of chemical inputs, so that the host-plant spectrum is determined by the distribution of these chemicals, which may act as feeding or oviposition deterrents. An assessment of the biochemical profiles of host-plants in terms of young and maturing leaves becomes essential. Age-correlated biochemical attributes tend to alter host suitability and consequently abundance of the thrips populations. In this connection, the need to identify the contact receptors on the maxillary and labial palps for better assessment of host-plant recognition becomes important. Probing of the leaf surface at different sites provides the needed chemical information with flavones, flavanones and flavanoid glycosides tending to act as feeding deterrents. Synergistic interactions of free amino acids with other compounds such as sucrose and other sugars tend to stimulate feeding. Since the concentrations of free amino acids are variable depending on age, species, variety and plant parts, an overall assessment becomes necessary. Understanding of such information

becomes important in view of individual species having different responses to host diversity. Variation in the age of plant tissues as well as their chemical composition tends to influence fecundity. Recent work on the dynamics of *T. tabaci*, *T. palmi* and *S. dorsalis*, which have spread extensively to the Far East and Japan, has shown that the intrinsic rate of increase of *T. palmi* differed with temperature during the cultivation period, the nature of increase from field experiment being 80–90% from that of the laboratory-reared[15]. Available records also indicate that *S. dorsalis* lays 40–70 eggs/female, with an average of 5–12/day. Similarly, populations of *T. tabaci* increase in considerable numbers during maturation or harvesting of vegetable crops. One cannot overlook the fact that throughout the season, composition of the crop changes, so that an understanding of vector dynamics in relation to seasonal changes in cropping system becomes important.

A further aspect of chemical ecology relates to induced resistance involving plant-mediated changes associated with thrips attack, resulting in the production of jasmonic and salicylic acids, which act as signals triggering naturally occurring chemical responses that protect the plant from natural invaders. Whether jasmonic and salicylic acid elicitors could be used as tools for controlling thrips and tospoviruses is a question for the future!

Molecular aspects of virus–thrips interactions

At the molecular level, the cellular events leading to viral multiplication and transfer mechanisms tend to be complex. Serodiagnosis could help in detecting disease outbreak and help plan management strategies well in advance[19]. Unfortunately, there are not many diagnostic tools available even for scientific studies. Another way of diagnosing TSWV is to use the RT–PCR technique[20], which is sensitive and can distinguish between different but similar tospoviruses. Detection of differences between tospoviruses is based on restriction enzyme digestions of the N-gene product from PCR[16], a technique which is time-consuming and requires laboratory equipment.

Transgenic crops engineered for major pests lead to reduction in pesticide applications, which might directly boost the survival and multiplication of some insects like thrips with vectorial capacity. Disease spread may take a heavier toll than pest damage in many important crops. Caution has to be exercised towards newer transgenic introductions, as there are unlimited chances for minor pests assuming a major status in the long run.

Conclusion

There is need for better appreciation of thrips–host plant interaction in terms of:

(a) Intra-specific diversity in thrips populations on various host plants all over the country.
(b) Nutritional diversity, nature of phenolics, and host shifts and ecological success.
(c) Rate of development of thrips on different hosts and fecundity.
(d) Sources of infection and build-up of thrips populations.
(e) Ecological succession, if any.
(f) Molecular aspects of thrips–virus interaction. The dependency of tospovirus on the biology of thrips is so great that it is surprising so little attention has been paid to the origin of this dependency[17].

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