FUNGI ASSOCIATED WITH HYPERTROPHIES CAUSED BY INFECTION OF CRUCIFERAE BY ALBUGO CRUCIFERARUM[1]

G. Allan Petrie[2] and T. C. Vanterpool[3]

Abstract

Over 20 species of fungi, including several pathogens of crucifers, have been found in association with hypertrophies of the inflorescence (stagheads) and stem and pod blisters produced on turnip rape (Brassica campestris), wild mustard (B. kaber) and false flax (Camelina microcarpa) by the white rust fungus, Albugo cruciferarum. The most prevalent associates of Albugo were Peronospora parasitica, Alternaria alternata, Fusarium roseum 'Acuminatum' and 'Equiseti', Alternaria raphani, A. brassicae and Cladosporium sp. Most of these were recovered on agar media following surface disinfestation of the hypertrophied tissue. Very few Albugo infected tissues failed to yield some secondary invaders, and five occasionally were isolated from a single staghead. The possible significance of these associations is discussed.

Resume

On a relevé la presence de plus de 20 espèces de champignons, y compris plusieurs pathogenes des crucifères, associées à des hypertrophies de l'inflorescence (bois de cerf) et à des boursouflures des tiges et des gousses de navette (Brassica campestris), de moutarde sauvage (B. kaber) et de caméline à petits fruits (Camelina microcarpa) produites par le champignon de la rouille blanche, Albugo cruciferarum. Avec Albugo, les isolats les plus fréquemment associés étaient Peronospora parasitica, Alternaria alternata, Fusarium roseum 'Acuminatum' et "Equiseti", Alternaria raphani, A. brassicae et Cladosporium sp. On a recouvré la plupart des pathogenes sur un milieu d'agar après désinfestation superficielle du tissu hypertrophié. Très peu des tissus infestés d'Albugo ont produit de parasites secondaires et on est quelquefois parvenu à en isoler cinq dans un même "bois de cerf". Les auteurs analysent l'importance éventuelle de ces associations.

Galls which form on plants in response to fungal infection appear to provide sites particularly favorable to the development of secondary microorganisms. For example, Xoch (5) reported the isolation of a dozen fungal species from the tissues of black knots produced on Prunus spp. by Dibotryon morbosum (Schw.) Theiss. & Syd. A few of these secondary invaders, notably Trichothecium roseum (Pers.) Lk., were active parasites of the stroma of D. morbosum, and appeared to check the development of the latter. Byler et al. (1, 2) also demonstrated that secondary fungi and insects were responsible for mortality of galls of pines caused by the rust Peridermium harknessii Moore.

Rust pustules may also serve as points of entry for other fungi. The infection of onion by Botrytis sp. via the sori of Puccinia asparagi DC. provides an early example of this (16). Invariably a rust pustule was found in the center of each Botrytis lesion and no infection by the latter occurred other than through sori of the rust. When invasion took place before the sori opened, the aecia failed to mature. A number of observers have reported infection of flax by various pathogens by way of pustules of Melampsora lini (Ehrenb.) Lév. secondary invaders included species of Fusarium (4, 6, 9, 10), Alternaria linicola Groves & Skolko (11, 12), Polyspora lini Laff. (11), and Septoria linicola (Speg.) Garass. (11). In certain years at least, fusarium stem canker of flax was invariably associated with rust infection (6), and Alternaria linicola stem lesions originated either in rust telia or leaf scars (12).

Observations over the last 15 years or more in western Canada have indicated that hypertrophies of the inflorescence (stagheads) of turnip rape (Brassica campestris L.) caused by Albugo cruciferarum S. F. Gray are ideal sites for copious spore production by various fungi. The association of Peronospora parasitica (Pers. ex Fr.) Fr.

---

[1] Contribution No. 547, Research Station, Agriculture Canada, Saskatoon, Saskatchewan, S7N 0X2.

[2] Plant Pathologist, Agriculture Canada, Saskatoon.

[3] Professor Emeritus, Biology Department, University of Saskatchewan, Saskatoon.

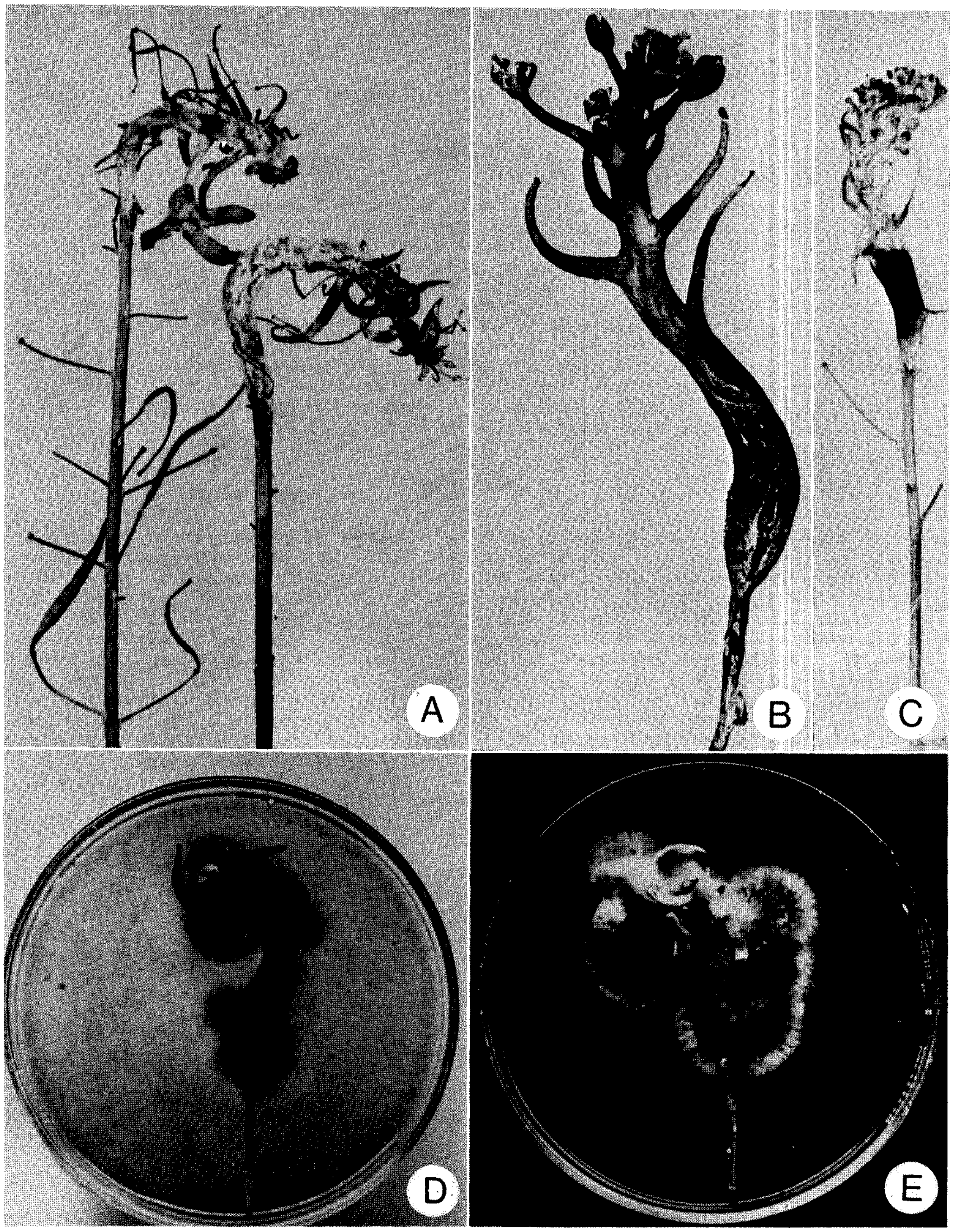

Figure 1. Fungi associated with *Albugo* hypertrophies (stagheads) on *Brassica campestris*. A) Downy mildew (*Peronospora parasitica*). B) *Alternaria brassicae*. C) *Peronospora* and *Alternaria* rp. developing on the same hypertrophy. D, E) *Alternaria* spp. growing from stagheads plated on nutrient medium.

with Albugo (Fig. 1 A, C) was perhaps the first commented upon (13). The appearance of black mold, mostly Alternaria alternata (Fries) Keissler, on stagheads (Fig. 1 B, C) is frequently striking following late summer or autumn rains (14), both in standing crops and those in swath. Less common associates of Albugo which we have reported previously are Fusarium spp., Alternaria raphani Groves & Skolko, A. brassicae (Berk.) Sacc. (7), and Mycosphaerella brassicicola (Duby) Lind. (8). Notes made by the junior author show that Fusarium spp. and Alternaria raphani had been found on hypertrophies collected at Meadow Lake, Saskatchewan, as early as 1959.

It was thought likely that other fungi commonly associated with stagheads went undetected during these observations. The fungi colonizing Albugo stem and pod blisters had also received scant attention. In addition, we had no indication of the effect that some or all of these associated species might have on A. cruciferarum. Therefore, in an attempt to obtain data relating to some of these considerations, the studies to be described were conducted over three growing seasons (1970-72).

Materials and methods

Stagheads and stem and pod blisters caused by Albugo, clipped from disease survey material collected in Saskatchewan largely in August of 1970, 1971, and 1972, were surface sterilized 2 min in 10% Javex (0.6% available chlorine) and plated on V8 juice agar containing 40 ppm rose bengal and 100 ppm streptomycin sulfate. Plating was done as soon as possible following collection. Plates were examined microscopically at 10 days and a record made of all microorganisms present. Many colonies were subcultured on V8 juice agar for further study.

Results

Prior to plating, a majority of the stagheads and stem and pod blisters bore no obvious indications that they harbored other fungi. Adjacent host tissue also usually appeared healthy. Nevertheless, there were few hypertrophies from which secondary organisms failed to grow. Initially, upon incubation profuse development of the mycelium and spores of secondary organisms occurred on the staghead or blister with no growth being evident on attached host tissue (Fig 1 D, E). On some stems, blisters were surrounded by prominent lesions (Fig. 2 A, B, C). It was evident from the symptoms and plating results that Alternaria had gained entry through the broken surfaces of the blisters and had initiated rather severe infections.

In all, 457 stagheads and 1841 stem and pod blisters were plated. All specimens came from Brassica campestris, with the exception of several pod blisters and stagheads from a collection of B. kaber (DC.) Wheeler from Eston, Saskatchewan, and a few stem blisters from plants of Camelina microcarpa Andrz. from near Pike Lake, Saskatchewan. The fungi most commonly isolated from the Brassica spp. are listed in Table 1. On B. campestris a greater variety of fungi occurred in stagheads than in blisters and usually each species was found in a higher percentage of stagheads than of blisters. On an average, approximately three fungi were found in a

Table 1. Fungi most commonly isolated from Albugo hypertrophies on Brassica spp.

Fungi isolated	Stagheads infected No.	Stagheads infected %	Stem and pod blisters infected No.	Stem and pod blisters infected %
Alternaria alternata	378	82.7	1,631	88.6
A. brassicae	66	14.4	633	34.3
A. raphani	153	33.4	278	15.1
Fusarium *roseum*	318	69.6	425	23.0
Cladosporium sp.	133	29.1	150	8.1
Stemphylium sp.	60	13.1	27	1.5
Epicoccum sp.	42	9.2	38	2.1
Gonatobotrys sp.	32	7.0	34	1.8
Rhizopus sp.*	28	6.1	5	0.3
Botrytis *cinerea*	14	3.1	1	0.1
Total no. pieces plated	457		1,841	

* And related genera.

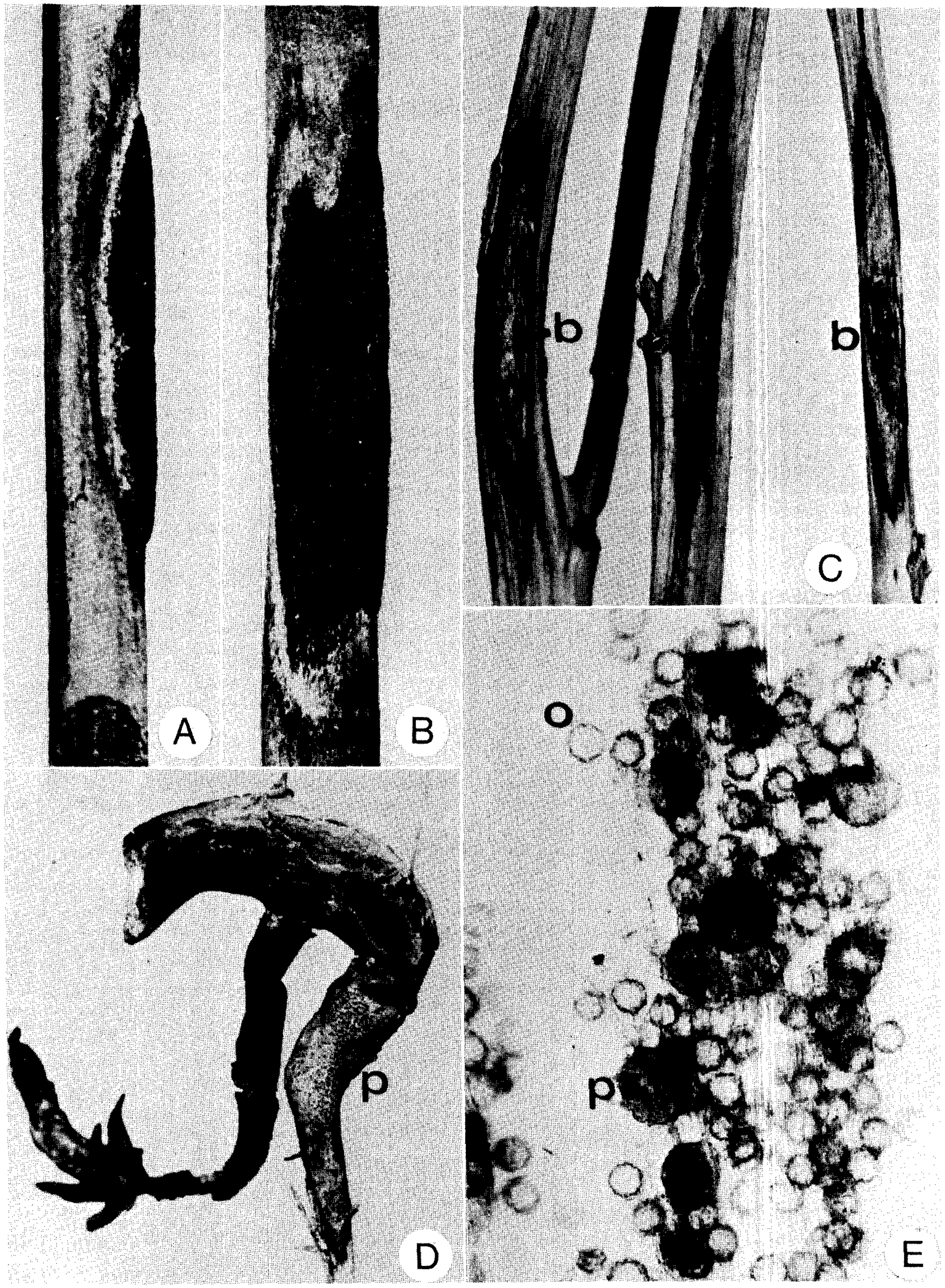

Figure 2. Fungi associated with *Albugo* hypertrophies. A, B, C) *Alternaria brassicae* developing on and around stem blisters (b). D) Pycnidia (p) of *Phoma* sp. on a staghead. E) Pycnidia (p) and oospores (o) in a scraping prepared from the staghead in D.

staghead and two in a blister. In a few individual fields, the average was closer to five fungi per staghead.

The most prevalent species in stagheads were Alternaria alternata, Fusarium roseum Lk. emend. Snyder & Hansen ('Acuminatum' and 'Equiseti'), A. raphani, A. brassicae, Cladosporium sp., Stemphylium sp., Epicoccum sp., Gonatobotrys sp., Rhizopus and related genera, and Botrytis cinerea Pers. All of these with the exception of A. alternata and A. brassicae were much less common on blisters (Table 1) but still were among the 10 most prevalent species. A. brassicae was recovered considerably more often from blisters than from stagheads, the reverse of what was found for A. raphani. This difference may result from the prevalence of moister conditions below the plant canopy where blisters occur, than at its top, where stagheads usually are found. Evidence from seed plating studies (15) has indicated that in drier years A. raphani is more common than A. brassicae.

The overall results were generally quite consistent from year to year. Comparing in each case the 2 years in which the greatest numbers of plating were made, Fusarium roseum was found in 69.4% and 65.5% of the stagheads and 22.0% and 25.9% of the blisters. The corresponding percentages for A. alternata were 90.4 and 79.5 for stagheads and 91.4 and 85.3 for blisters. When data from the different fields were compared, considerable variation was noted in the percentages of hypertrophies from which any given species was isolated. For example, in 1972, percentages of stem blisters harboring A. raphani ranged from 1.5% to 66.7%, with an average for all fields of 13%. For Fusarium roseum the range was 0 to 84.3%, with an average of 25.9%. A. alternata was much more uniformly distributed, occurring in over 80% of the stem blisters in all fields but two.

In addition to the 10 species listed in Table 1, at least as many more were found relatively less frequently. These included Acremoniella sp., Alternaria of the linicola type, Arthrinium sp., Chaetomium sp., Curvularia sp., Gliocladium roseum (Lk.) Bainier, Plenodomus lingam (Tode ex Fr.) Höhn., Pleospora herbarum (Fr.) Rabh. and miscellaneous pycnidial fungi, principally Phoma herbarum Westend. Mycosphaerella brassicicola was observed on a few stem blisters before they were plated but could never be cultured from the material due to the presence of more aggressive species. Of those fungi which could not be identified to genus, the most common was a white, rapidly-growing non-sporulating form.

The Brassica kaber specimens yielded Alternaria alternata, Cladosporium sp., A. raphani and the white, non-sporulating fungus. Fusarium and Stemphylium occurred sporadically. Several cultures of A. alternata, A. brassicae and Fusarium roseum and a few of Epicoccum sp. and Cladosporium sp. were obtained from the Camelina stem blisters.

A few unreported observations made by the junior author prior to 1970 may also be noted. In 1968, Erysiphe polygoni DC. ex Mérat was found in association with Albugo at Saskatoon. Also in that year, Fusarium roseum 'Avenaceum' was isolated from hypertrophies. In 1960, three isolates of Phoma were obtained from stagheads collected at Brooksby, Saskatchewan, and in 1962 an unusual species of Phoma moderately pathogenic to rape seedlings was isolated from material received from Fort Vermilion, Alberta (Fig. 2D, E).

Discussion

The plating method used here for the determination of the fungal associates of Albugo was undeniably imperfect, in that some important species could not be recovered. The bacterial flora was not studied. However, if observations made in the field and verified by microscopic examinations are combined with the plating data, it is felt that a reasonably complete picture may be obtained of the fungi to be found most frequently in hypertrophies. These would appear to be Peronospora parasitica, Alternaria alternata, Fusarium roseum, A. raphani, A. brassicae and Cladosporium sp. The association of the first of these with Albugo is so intimate that the two fungi have long been considered to constitute a single disease complex.

The advantages afforded secondary invaders of Albugo hypertrophies for spore production and dissemination are quite apparent. Other advantages may also accrue. Stem blisters are obviously an important avenue for invasion of the plant by pathogenic Alternaria species (Fig. 2) and other fungi including Fusarium, and, occasionally, Plenodomus lingam. Much work remains to be done on the mutual interactions of Albugo and associated microorganisms. Inhibitory substances have been leached from stagheads (unpublished data) but the effects of these on other fungi are unknown. Some of the Alternaria species considered here are known to produce toxins (3) and these could conceivably have an adverse effect on survival of Albugo. Work is continuing in an attempt to clarify some of these points.

Acknowledgments

The authors express their appreciation to Mrs. Jean Key and Mrs. Marjorie Richardson for technical assistance.

Literature cited

1. Byler, J. W., F. W. Cobb, Jr., and J. R. Parmeter, Jr. 1972. Effects of secondary fungi on the epidemiology of western gall rust. Can. J. Bot. 50: 1061-1066.

2. Byler, J. W., F. W. Cobb, Jr., and J. R. Parmeter, Jr. 1972. Occurrence and significance of fungi inhabiting galls caused by Peridermium harknessii. Can. J. Bot. 50:1275-1282.

3. Degenhardt, K. J. 1973. Alternaria brassicae and A. raphani: Sporulation in culture and their effects on yield and quality of rapeseed. M.Sc. thesis, University of Alberta, Edmonton, 91 p.

4. Jaczewski, A. A. 1928. Concerning the diseases of oil seed plants. (In Russian.) Oil and Fats Producing Industry, 1928, 4:1-11. Abstr. in Rev. Appl. Mycol. 8: 381-382, 1929.

5. Koch, L. W. 1934. Investigations on black knot of plums and cherries. II. The occurrence and significance of certain fungi found in association with Dibotryon morbosum (Sch.) T. & S. Sci. Agr. 15:80-95.

6. Machacek, J. E, and W. L. Gordon. 1943. Pages 25-26 in 22nd Annu. Rep. Can. Plant Dis. Surv. 1942.

7. Petrie, G. A., and T. C. Vanterpool. 1966. Diseases of rape, mustard and cruciferous weeds in the prairie provinces. Can. Plant Dis. Surv. 46: 117-120.

8. Petrie, G. A., and T. C. Vanterpool. 1968. Diseases of crucifers in Saskatchewan in 1967. Can. Plant Dis. Surv. 48: 25-27.

9. Sackston, W. E. 1949. Pages 24-28 in 28th Annu. Rep. Can. Plant Dis. Surv. 1948.

10. Vanterpool, T. C. 1944. Flax diseases in Saskatchewan in 1943. Can. Plant Dis. Surv. 23:21-23.

11. Vanterpool, T. C. 1949. Flax diseases in Saskatchewan in 1948. Can. Plant Dis. Surv. 28:22-24.

12. Vanterpool, T. C. 1953. Flax diseases in Saskatchewan in 1952. Can. Plant Dis. Surv. 32: 31-32.

13. Vanterpool, T. C. 1960. Rape diseases in Saskatchewan in 1958. Can. Plant Dis. Surv. 38: 37-38.

14. Vanterpool, T. C. 1960. Rape diseases in Saskatchewan in 1959. Can. Plant Dis. Surv. 39: 30-31.

15. Vanterpool, T. C. 1963. Rape diseases in Saskatchewan in 1963. Can. Plant Dis. Surv. 43: 212-214.

16. Walker, J. C. 1921. Rust of onion followed by a secondary parasite. Phytopathology, 11:87-90.